Diet choice, foraging behaviour and the effect of predators on feeding in the three-spined stickleback (*Gasterosteus aculeatus* L.).

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ABSTRACT

This thesis describes a study on diet choice, foraging behaviour and the effect of predators on feeding in three-spined sticklebacks (Gasterosteus aculeatus L.) from three sites in Scotland.

The profitability values of prey items of various types and sizes were determined in a simple laboratory experiment, by dividing the mean energetic contents of each prey type by the mean handling time for that prey type. Profitability increases with fish size and with prey size (for some but not all prey types). Handling time increases with the amount of food in the stomach. Both fixation and handling times are an increasing function of prey size and a decreasing function of fish size. Both fixation and handling times differ from one prey type to another.

The effect of experience on various components of feeding on natural prey in sticklebacks was investigated by testing two groups of fish, one reared with only frozen brine shrimps and the other caught in the wild where a wide range of food was available to them. Feeding latency, fixation and handling times and the number of grasps required before natural prey could be eaten are lower in fish with experience with natural food. However, this reduced feeding efficiency in naive fish disappears within just few days of experience with natural prey.

Differences between populations of sticklebacks exposed in nature to different invertebrate fauna in the efficiency (the net energy gain per unit time) of feeding on zooplankton or benthos were investigated. The results suggest that sticklebacks from areas with abundant zooplankton feed more efficiently on this kind of food than sticklebacks from areas of low zooplankton but high benthos which in turn feed more efficiently on benthos (this difference is not significant). Both groups of fish feed more efficiently on zooplankton compared to benthos but this difference is more marked in the case of fish derived from areas with abundant zooplankton.

The role of prey profitability in food choice was investigated by using two different sizes of sticklebacks choosing between prey of different profitabilities in a series of laboratory tests. Fish of

both sizes do not always choose the more profitable of two prey items. In the over all comparisons, only in 33.3% and 66.6% (for smaller and larger sized fish respectively) of the cases the more profitable prey type was selected. The role of the various visual stimuli that differentiate the natural prey types (speed and type of movement, colour, shape and size) in food selection was investigated systematically. The results suggest that sticklebacks differentiate among various configuration of each stimulus and that the different kinds of stimuli differ in their importance as determinants of prey choice. The results obtained when sticklebacks were offered a choice between prey of different profitability (see above) arose because the fish choose the prey on the basis of one or more preferred visual cues. In some cases, these cues lead the sticklebacks to the most profitable prey and in others they do not do so.

The distribution of potential food was studied on three different types of substrates in Loch Lomond over the summer of 1985. The abundance of different food types varies according to the kind of substrate, even within few yards, and from month to month within a single season. Food selection in sticklebacks was also studied in relation to these variations in prey availability. Sticklebacks select zooplankton rather than benthos, but this selection is influenced by the type of substrate above which the fish are feeding and by time of year. Generally, the selective feeding under natural conditions can be explained in terms of the set of preferred visual cues identified in the laboratory experiments (see above), but in this case these cues lead the sticklebacks to the most profitable prey types (zooplankton). Prey size selection was also investigated by comparing prey size distribution in the diet with prey size distribution in the habitat. Sticklebacks select larger items of zooplankton and smaller items of benthos.

A comparison of the natural diet of three-spined sticklebacks and ten-spined sticklebacks from Loch Lomond during the summer of 1985 suggests that no food competition should occur since, at this time of the summer, these two species have different diets and show positive selection for different prey types.

Two different habitats of sticklebacks (Loch Lomond and Balmaha Pond) were studied for the food and predators available and the

sticklebacks from these two sites were studied for food preference, habitat use and morphology. The results suggest that, even though both fish groups show a positive selection for zooplankton, they have different feeding habits (which reflect the type of food available), prefer different habitats and differ in the gape size and number, length and spacings of the gillrakers. Defence apparatus (dorsal and ventral spines) also differ between the two fish groups, with Loch Lomond fish having longer spines than Balmaha Pond, reflecting the estimated risk of predation at the study sites.

The effect of predation risk on the feeding behaviour of sticklebacks was investigated in both laboratory and field experiments. The time to start feeding, the ability to discriminate in favour of the most profitable prey, the amount and size of food eaten and vertical distribution in the habitat can all be affected by predation threat. The effect of local predation risk on feeding behaviour in sticklebacks was also investigated by using fish from two local populations of contrasting levels of predation risk. It is found that, although food intake is suppressed by the presence of a predator in sticklebacks from both sites, diet selection is more markedly impaired in those from high predation sites.

The results obtained in this programme of research are discussed in the light of current developments of Optimal Foraging Theory and of the existing literature on feeding behaviour of fish.

CHAPTER 1

INTRODUCTION

1.1 THE BIOLOGY OF STICKLEBACKS

1.1.1 Taxonomy

Three-spined sticklebacks (Gasterosteus aculeatus) are small teleost fish (2.5-10 cm, adults standard length) of the family Gasterosteidae. They are widely distributed in fresh, brackish and salt waters of the middle and high latitudes of the Northern hemisphere. Three common morphs of this species can be recognized (sometimes referred to as subspecies); these are classified mainly according to the number of lateral bony plates (Wootton 1984). The Trachurus form is usually anadromous and represents the completely plated morph with 30-35 lateral plates run on each side of the body. The Semiarmatus form is partially plated, with 12-30 lateral plates occurring in two groups, one group on the anterior section of the body separated from a second group forming the caudal keel. The Leiurus form normally occurs in fresh water and represents the low plated morph, with 0 to 14 lateral plates on the anterior section of the body. All the sticklebacks used in the present study belonged to the low plated (Leiurus) morph.

1.1.2 Feeding behaviour

Sticklebacks are predominantly carnivorous feeders (Wootton 1976) and their small size restricts the range of food that they eat to small invertebrates such as zooplankton and certain small benthic species. Although their usual food items are small in size, three-spined sticklebacks are capable of consuming prey whose maximum body width is nearly equal to their jaw width (Burko 1975). Sticklebacks

detect their prey visually, so they have relatively large eyes. Adult sticklebacks can detect a 10 mm long Asellus at a maximum distance of 44 cm in clear water and at 26 cm in turbid water (Moore and Moore 1976 a). The physical characteristics of the prey such as movement, colour contrast and size, therefore, play an important role in prey detection (Wootton 1984).

The foraging sequence in three-spined sticklebacks is fully described by Beukema (1968). Briefly, the fish initiate a search, which may eventually lead to discovering the prey. Once a prey is discovered, it will be approached, fixated and inspected, pursued (if the prey tries to escape capture), grasped and swallowed or (occasionally) rejected. The feeding sequence may be broken at any point, depending on factors associated with the prey (eg. palatability, escape ability) and/or the predator (eg. hunger).

1.1.3 Predators and anti-predator defence

Adult sticklebacks are potential prey of a wide variety of predators including fish (eg. trout, Salmo trutta; pike, Esox lucius), birds (eg. kingfisher, Alcedo atthis; heron, Ardea cinerea), mammals (eg. mink, Mustela vison, otter, Lutra lutra), and reptiles (eg. garter snakes, Thamnophis couchi hammondi). Due to their small size, fry, larvae and eggs of sticklebacks have an even wider range of predators, adding invertebrate predators such as leeches (Haemopsis marmorata), dragonfly nymphs (Odonata), waterbugs (Hemiptera) and Dytiscus sp. larvae and adults (Coleoptera).

Sticklebacks defend themselves against predators by a range of mechanisms which can be classified into primary and secondary defence (Edmunds 1974). Primary defence mechanisms are those that act regardless of whether a predator is present or not, acting to reduce the possibility that the fish will be detected by predators. Sticklebacks have the ability to adapt their colour to match a part of their environment and thus become inconspicuous to predators. For example, when a stickleback is transferred from a dark to a pale substrate, its usual dark dorsal surface pales within few seconds and the lateral strips pale within few minutes.

Secondary defence mechanisms include behavioural and morphological adaptations which increase the chance of sticklebacks escaping predation once detected, or of surviving the encounter once captured. When it detects a predator, a stickleback may stop or approach the predator, inspecting and identifying the danger (Magurran et al. 1985) before retreating or jumping away in an unpredictable direction, usually to hide in any available refuge. Alternatively, the stickleback may freeze, becoming completely motionless except for a slight opercular movement (Benzie 1965, Wootton 1984).

With exception of certain spine- and plate-deficient populations of three-spined stickleback (Giles 1983, Campbell 1985), morphological adaptations to predation risk include body armour, consisting of dorsal & ventral spines and lateral bony plates. The presence of the spines increases the effective dimensions of three-spined sticklebacks (by up to 75%) so that predators find difficulty in handling and swallowing them. The presence of anterior lateral bony plates supports and stabilizes the erected spines (Wootton 1984).

Thus, the dorsal and ventral spines tend to be larger and the number of anterior lateral plates tends to increase (up to 7) in stickleback populations which are sympatric with predators (Reimchen 1983). A part of this project is aimed **(aim 1)** at investigating the defence apparatus in sticklebacks from two sites in order to identify two populations of contrasting levels of predation risk. These are needed to study the effect of local predation pressure on feeding behaviour of sticklebacks.

1.1.4 The diet of three-spined sticklebacks

The diet of three-spined sticklebacks has been widely studied (eg. Hynes 1950, Maitland 1965, Manzer 1976, Allen and Wootton 1984, Ukegbu 1986). This diet consists mainly of zooplankton (principally cladoceran and copepods), insect nymphs (ephemeropteran, plecopteran, and corixids), benthic crustacea (Asellus and Gammarus), insect larvae and pupae (dipteran, coleopteran), molluscs and eggs (of both invertebrates and sticklebacks themselves).

Allen and Wootton (1984) studied the seasonal variation in the diet of sticklebacks living in Llyn Frongoch (Wales) and found that copepods were prominent in the diet in spring and autumn. Ephemeroptera nymphs and, to lesser extent, Chironomid pupae and stickleback eggs were prominent in the diet during summer. Chironomid larvae and Ostracoda were important in the diet throughout the year.

In order to determine whether these changes in diet are a result of changes in the density and availability of the prey or simply a result of changes in the selectivity of sticklebacks, detailed studies of these two parameters are required. The degree of selectivity shown

by fish to a particular prey type can be determined by direct comparison between the proportions of a food type in the diet and in the environment, at the same time and location. Manzer (1976) reported that three-spined sticklebacks living in Great Central Lake, Vancouver Island (British Columbia), fed predominantly on Bosmina during April but gradually switched to Holopedium, which dominated the diet by October. By using the data available on the food distribution for the same site (LeBrasseur and Kennedy 1972), Manzer concluded that such changes in the diet of stickleback were a result of changes in the abundance of the food in the environment. However, in Manzer's study, there was time and space variation between sampling the fish and sampling the food available. Therefore, the food available to the fish whose stomachs he analysed was not precisely monitored. One aim of this thesis **(aim 2)** is to relate the diet and food selection of three-spined sticklebacks to the availability of potential food types in the sites where the fish feed as these food types vary in time and space. Studying the variability of the potential food available to sticklebacks may give some insight into the kind of foraging decisions that sticklebacks must make when foraging in their natural habitats.

1.1.5 Population differences in diet and morphology of feeding apparatus

Stickleback populations may differ in their diet, and such differences usually related to the differences in the food availability. Ukegbu (1986) studied the diet of three populations of three-spined sticklebacks in the region of Glasgow (Scotland) and reported that, in two populations (River Luggie and River Kelvin), sticklebacks fed mainly on benthos (dipteran larvae and Asellus)

whereas the third population (Aurs Burn) fed, in addition to dipteran larvae, on zooplankton.

Differences in diet of the three-spined sticklebacks may also be related to differences in habitat choice and specialization on the food available in that habitat. Larson (1976) has recognized two distinct forms of sticklebacks in Paxton Lake (British Columbia) that coexist in different regions of the lake, open water and littoral. These two forms of stickleback (called 'limnetic' and 'benthic' sticklebacks respectively) specialize on different food types, i.e. the food available in their respective habitats. Thus, limnetic sticklebacks feed predominantly on the small organisms inhabiting the water body (zooplankton) whereas benthic sticklebacks feed predominantly on larger prey items that inhabit the lake bed (benthos). This specialization on different food may result in variation in the morphology of feeding apparatus (mouth, eye, and gillrakers) of stickleback populations. Thus, limnetic sticklebacks are characterized by narrower snouts and larger protrusive eyes. In addition, they have more numerous, closely spaced and longer gillrakers which are thought to enable them of feeding on zooplankton. In contrast, benthic sticklebacks are characterized by broader snouts, non-protrusive eyes, and fewer, coarsely spaced, and shorter gillrakers which are sufficient for feeding on benthos. Many studies (eg. Bentzen and McPhail 1984, Lavin and McPhail 1986) have been carried out on the ability of limnetic and benthic sticklebacks to handle a given food type and found that each of these two forms is more efficient at feeding on the food found in its natural habitat. Benthic sticklebacks (which feed on larger prey items), for example, consume a given benthic prey within shorter times than limnetic sticklebacks. This method of measuring the feeding efficiency clearly

does not take into account the actual energetic gain obtained by the fish.

So the work on the differences between stickleback populations has so far involved either quantitative accounts of the food eaten or studies on feeding efficiency (which has been dealt with in general terms) in relation to morphology of the feeding apparatus. Thus, part of this thesis (aim 3) studies, in further detail, differences in food selection, habitat use and feeding efficiency between sticklebacks from two different localities, and relate this to the morphology of the feeding apparatus of the fish.

1.2 OPTIMAL FORAGING THEORY AND FISH FEEDING

Optimal foraging models assume that efficient foraging increases fitness, and seek to determine those patterns of foraging that maximize some measures of food intake. In this framework, foraging animals must decide which food types to eat, which patch to feed in, and how long to spend in each patch (Krebs 1978). Considerable attention has been given to the first decision concerning prey choice, and emphasis has been placed on the ultimate strategic 'goal' of prey selection.

While consuming their food, foragers gain an energetic return from which a certain amount has to be traded-off against the various costs of finding and handling the food. The most commonly discussed costs are the time and energy required during foraging, but reduced opportunity for other activities is also important. So, the net energy return of the food is an increasing function of the energetic content of the prey and a decreasing function of the time spent in

obtaining such prey. This relationship is called 'profitability' and can be defined as the total energetic content divided by the time taken to handle the prey (Pyke et al. 1977, Townsend and Hughes 1981). Thus, classical Optimal Foraging Theory assumes that animals should select a prey that maximizes the profitability of the food.

However, recent developments of Optimal Foraging Theory recognize that energy maximization may not be the only 'goal' of foraging. Other nutrient elements, for example, may be important as well as energy (Goss-Custard 1977); the ratio of protein to fibre may be more important than energy for monkeys, Alouatta palliata (Milton 1979). However, the energy maximization assumption is more realistic for fish which feed on animal food, for their diet will usually contain an adequate supply of other nutrients (Townsend and Winfield 1985).

Classical optimal foraging models predict that when confronted with more than one prey type, foragers should consume those prey categories that maximize the energy intake per unit time (i.e. the most profitable prey), and reject those items with lower profitability. So, as the encounter rate with the most profitable prey declines, foragers should expand their diet range further to include the second most profitable prey categories and so on.

Many empirical studies have shown this to be the case. Werner and Hall (1974), for example, offered bluegill sunfish (L. macrochirus) 3 size classes of Daphnia magna, all of which took similar times to be eaten (1-2 sec) and therefore the profitability increased with increasing prey size. The experiment was performed at 3 prey densities - low, intermediate, and high. The result indicated that at low prey density the fish showed no preference between the three size

classes of Daphnia, but at intermediate prey density, the fish ate more of the medium and the large prey, i.e. selecting against the smaller prey. Furthermore, by increasing the prey density further, the fish ate the largest prey size class almost exclusively, thus selecting against small and medium sized prey. The behaviour of bluegills was consistent with the predictions of the Optimal Foraging Theory in that they selected the most profitable prey at high prey density and broadened their diet to include the less profitable prey as the prey density declined.

Gibson (1980) observed that when offered two size classes of Daphnia magna at low prey density, three-spined sticklebacks chose the Daphnia that appeared larger (irrespective of their absolute sizes), so that some smaller prey were included in the diet. At high prey density, however, the fish did not specialize on the largest Daphnia. This behaviour of sticklebacks was in accordance with the prediction of Optimal Foraging Theory because at high prey density, search time for large prey was not short enough for the rate of food intake to be maximized by ignoring smaller prey items.

Most laboratory tests on diet choice in fish have been carried out using just one prey species whose individuals differ presumably only in prey size. The decision of the fish to choose their prey is therefore based on simple criteria. In nature, fish such as sticklebacks encounter and consume various species of prey and thus the use of just one prey type does not reflect the choice of fish feeding in the natural environment. One aim of this study **(aim 4)** is to determine the costs and benefits of feeding on various natural-prey types by three-spined sticklebacks, and to investigate the role of prey profitability in diet choice when selecting between more than one prey type.

1.3 COMPLICATIONS OF OPTIMAL FORAGING THEORY

1.3.1 Optimal Foraging Theory and learning

Classical Optimal Foraging Theory assumes that foraging animals respond to their prey in a constant way throughout their foraging bouts; i.e. the efficiency of foragers, and hence energetic gain of the food does not vary with experience. This is unlikely to be the case since, in natural environment, prey availability is changeable and foragers must modify their behaviour accordingly. This modification in the behaviour of foragers can come about by learning process.

In addition to learning about the changes in the environmental conditions, foragers may learn how to manipulate and handle their prey. Ringler (1979) tested the performance, as the daily energetic intake (kj), of brown trout (S. trutta) feeding on drifting prey (freshly thawed brine shrimp, crickets, and meal worms) in an experimental stream. Food items were introduced into the experimental stream for short periods repeated at daily intervals for several days. He found that the performance of the fish increased dramatically with successive feeding trials with the result that within just 5 days it increased more than two fold. Winfield et al. (1983) tested the performance of bream (Abramis brama), measured as the number of prey (Cyclops vicinus) swallowed divided by the number of approaches made to the prey. The test was run in daily trials (15 min each) for 5 days. They found that the fish constantly increased their efficiency (up to 72%) over the first 3 days of the trials, after which this efficiency stabilized.

Only few studies have investigated the effect of experience on feeding behaviour in three-spined sticklebacks and such effect has been usually dealt with in very general terms. Beukema (1968) has shown that sticklebacks feeding on unfamiliar prey (Drosophla) increase their average reaction distance (the distance from which the fish respond to the prey) as they become experienced with the prey. This improvement in reaction distance is attributed to learning specific features of the prey and forming 'searching images' (Lawrence and Allen 1983). Thomas et al. (1985) have shown that sticklebacks have the ability to learn to recognize which is the highly yielding site and exploit it exclusively.

So, increased experience with the prey may alter the balance of the costs and benefits of foraging. An aim **(aim 5)** of this project is to investigate in more detail how experience with food can affect various components of foraging in three-spined sticklebacks.

1.3.2 Optimal Foraging Theory and 'rules of thumb'

Optimal Foraging Theory describes how foragers should choose their food, if they are designed by natural selection to maximize the net energy of foraging, without reference to the proximate mechanisms by which foragers achieve this 'goal'. Thus, classical Optimal Foraging Theory clearly assumes that foragers are able to recognize the profitability order of the food items and select the most profitable of them. This is found to be the case in newly hatched snakes (genus Thamnophis) feeding on water extracts of a variety of animal species in a simple laboratory experiment (Burghardt 1969). However, in

complex, natural habitats where the profitability value of the food is changeable, optimal foragers would need to sample the food available regularly in order to collect the information required, and then memorize such information at least for a foraging bout. This procedure would cost the foragers energy and tend to lower the value of the diet in respect to the short-term optimum, because they must sample food of varying quality to obtain the required information. In addition, the requirement of processing such information places great demands on the behavioural capacity of foragers that include a large number of prey types in their diet, especially if the relative profitability of the food varies with time. It has been suggested, therefore, that instead of making complex 'calculations' of the profitability, predators may select prey on the basis of a set of relatively easily distinguished stimuli which in most circumstances, correlate with profitability. By doing so, animals solve their foraging problems and may approximate to the solution predicted by Optimal Foraging Theory. Such decision mechanisms (or 'rules of thumb') usually, but not invariably, lead foragers to the most profitable prey items as specified by the Optimal Foraging Theory.

Hubbard and Cook (1978) showed that the parasite Nemeritis canescens searching for hosts (Ephestia cantella) in an area with patches of varying number of hosts allocates its searching time according to host density. Such behaviour was in accordance with the prediction of Optimal Foraging Theory. Waage (1979) studied the mechanisms underlying this behaviour of N. canescens and concluded that it was a simple decision based on habituation to host scent.

Mazur (1981) has provided evidence that food choice is not necessarily governed by principles of optimization theory. White Carneaux pigeons exposed to a choice between two food resources shift

their diet choice in a direction that decreases the rate of reinforcements. However, even when the proximate rules do not lead foragers to maximize the net energy intake as predicted by the classical models of Optimal Foraging Theory, it should not be regarded as 'suboptimal' strategy. Foragers may still maximize the payoff if various foraging constraints are taken into account. Janetos and Cole (1981) have suggested that the cost of increasing the performance above a specific level may exceed the extra benefits that might be gained by doing so.

Few studies have been carried out in an attempt to characterize the visual cues used in prey selection by sticklebacks (eg. Meesters 1940, Ohguchi 1981) but such studies have not involved enough stimuli and no firm conclusion has been drawn. One aim of this study **(aim 6)** is to characterize in detail the visual cues used by three-spined sticklebacks to select their food under both simple, laboratory conditions, and in a more complex, natural environment, and to see if these cues lead the fish to the most profitable prey.

1.3.3 Optimal Foraging Theory and predation risk

Optimal Foraging Theory does not take into account the changes in behaviour of foragers due to predation risk. It assumes that foragers should choose the most profitable prey regardless of the risk they face. Foragers of various species, facing risk of death by predators, should be willing to sacrifice energy in order to avoid this risk, especially if the two tasks, optimal feeding and predator avoidance, can not be performed simultaneously. Animals must maintain

at least a minimum energy intake, so in such a situation, a trade-off between energetic return from foraging and predation risk (rather than simply maximizing the food intake or terminating foraging) is expected. The optimal compromise in this case is thought to be a balance between efficient feeding and predator avoidance. Thus, the value of alternative food available (Cerri and Fraser 1983) and the level of predation risk (Fraser and Huntingford 1986) can have a substantial influence on foraging decision when a compromise between optimal feeding and predator avoiding is to be made.

Thus, predation risk can influence the balance between benefits and costs associated with a particular mode of feeding. Werner et al. (1983), for example, placed three size classes of bluegill sunfish (Lepomis macrochirus) in a natural pond divided into two halves, one of which contained largemouth bass (Micropterus salmoides), which can feed efficiently only on the smallest size of bluegill. In the absence of the predator, all three size classes of bluegill occurred in the open water habitat, which is the most profitable one. When predators were present the smallest, most vulnerable bluegill, but not the other size classes, restricted their foraging to the vegetated habitat which is less profitable. As a consequence, their growth rate was greatly reduced. This indicates that the small-sized prey fish avoided the more profitable, but dangerous habitat, in favour of less profitable but safer one and paid a growth cost.

The systematic trade-off between predation risk and optimal foraging can be influenced by the predation conditions at the sites from which the foragers are derived. Magurran (1986) studied the effect of a predator (pike, Esox lucius) on foraging behaviour of minnows (Phoxinus phoxinus) from two populations, one from a high predation risk site and the other from a low predation risk site. She found

that minnows from the high risk site kept greater distance between themselves and the predator and were less likely to recommence foraging than those from the low risk site. Fraser and Gilliam (1987) studied the effect of a predator (Aequidens pulcher) on feeding behaviour of guppies (Poecilia reticulata) and Hart's rivulus (Rivulus harti) from two sites of contrasting levels of predation risk. In this case, in contrast, fish derived from areas of high predation risk fed at consistently greater rate and maintained their feeding rate for longer time in the presence of a predator than fish derived from areas of low predation risk.

Although the classical work on the impact of predation risk on foraging decision has used three-spined sticklebacks as subjects (Milinski and Heller 1978), there is actually rather little work on just how three-spined sticklebacks make the trade-off between food and predation risk. Milinski and Heller conducted a laboratory test in which hungry three-spined sticklebacks were allowed to choose between various densities of Daphnia magna. The sticklebacks changed their choice of feeding area from a high density of Daphnia, which provided higher feeding rates for hungry fish, to a lower density area which provided lower feeding rates but increased the ability of fish to detect predators while continuing feeding.

In another laboratory test, Milinski (1985) allowed individual three-spined sticklebacks to feed in the presence of a large cichlid (Oreochromis mariae) behind a glass partition. The food (Tubifex worms) was placed in deep rings on the bottom of the experimental tank so that once the fish started feeding they lost sight of the predator. After they were exposed to the predator, three-spined sticklebacks picked up individual Tubifex which were furthest away from the

predator and fed more slowly than the fish which were not exposed to the predator. By doing so, the fish arrived at a compromise between feeding efficiency and predator avoidance.

Most existing work on the effect of predation risk on feeding behaviour of three-spined sticklebacks has so far been done in the laboratory under extremely simple conditions and involved just a single prey type. In addition, few studies, and all in the laboratory, have been carried out on the effect of local predation pressure on subsequent foraging behaviour in fish, and none has been carried out on sticklebacks. Moreover, the effect of predation risk on habitat use by sticklebacks has not been established. Thus, an aim of this thesis **(aim 7)** is to extend the existing work on this subject and quantify the effect of predators on the foraging behaviour of sticklebacks feeding on more than one prey type, in the field as well as in the laboratory. The effect of local predation pressure on behaviour and the effect of a predator on habitat use are also investigated.

1.4 SUMMARY OF AIMS

The laboratory and field experiments described in this thesis are designed with the following aims in mind:

1. To identify two stickleback populations of contrasting level of predation risk in order to use them in a study of the effect of local predation pressure on feeding behaviour **(aim 1, Chapter 6).**

2. To measure the variability, in time and in space, of the potential food of sticklebacks, and to determine how feeding habit and food selectivity of sticklebacks vary accordingly **(aim 2, Chapter 5).**

3. To study the differences in feeding efficiency, food selection and habitat use between stickleback populations and relate these to the morphology of the feeding apparatus of the fish **(aim 3, Chapters 3 & 6).**

4. To measure the costs and benefits of feeding on natural prey by sticklebacks and to investigate whether sticklebacks choose their food according to the profitability value of the prey **(aim 4, Chapters 3 & 4).**

5. To determine in detail the effect of experience on feeding efficiency in sticklebacks **(aim 5, Chapter 3).**

6. To characterize the proximate cues that sticklebacks use to select their food under both laboratory and field conditions **(aim 6, Chapters 4 & 5).**

7. To quantify the effect of predators on the foraging behaviour of sticklebacks feeding under laboratory conditions on more than one prey type and under field condition on an array of prey types. To investigate the effect of local predation pressure on subsequent foraging behaviour, and the effect of a predator on habitat use **(aim 7, Chapter 7).**

CHAPTER 2

GENERAL METHODS

2.1 General description of the study sites

The experiments described in this thesis have been carried out on fish from 3 locations in Glasgow area, Loch Lomond (NS 373 957, National Grid Reference), Balmaha Pond (NS 422 910), and the River Kelvin (NS 563 688). The characteristics of these study sites are presented below:

Loch Lomond

Loch Lomond is a large mesotrophic fresh-water lake with a length of 36 km, a width of 7 km at the widest point, and a maximum depth of a 190 m. Samples taken during summer 1984, 1985 and 1986 have revealed high density of both zooplankton (up to 147 individuals/l, June 1985) and benthos (up to 5150 individuals/m^2, August 1985).

Compared to other Scottish lochs, Loch Lomond has a highly diverse fish fauna, where 14 species, other than three-spined stickleback (Gasterosteus aculeatus), have been recorded (Maitland et al. 1981). Fish species which prey upon adult sticklebacks in the Loch include pike (Esox lucius), perch (Perca fluviatilis), brown trout (Salmo trutta) and eel (Anguilla anguilla). Other fish species include salmon (Salmo salar), powan (Coregonus lavaretus), roach (Rutilus rutilus), minnow (Phoxinus phoxinus), stone loach (Noemacheilus barbatulus), ten-spined stickleback (Pungitius pungitius), flounder (Platichthys flesus), sea lamprey (Petromyzon marinus), river lamprey (Lampetra fluviatilis), and brook lamprey (Lampetra planeri). Ruffe (Gymnocephalus cernua) have been frequently caught during fishing trips to the Loch in summers of 1985 and 1986.

Birds are also common around Loch Lomond, with some 200 species being recorded (Richmond 1974), some of which are potential predators of sticklebacks (Giles 1981). These include heron (Ardea cinerea), red-breasted merganser (Mergus serrator), black-headed gull (Larus ridibundus), great-crested grebe (Podiceps cristatus), little grebe (Tachybaptus ruficollis), tufted duck (Aythya fuligula), goldeneye (Bucephala clangula), common gull (Larus canus), common tern (Sterna hirundo), and artic tern (Sterna paradisaea).

Balmaha Pond

Balmaha Pond is a small pond situated at c. 200 m east of Loch Lomond. Its dimensions are roughly 40x25 m and a maximum depth of 1.5 m. It is dominated by thick cover of submergent and emergent aquatic-vegetation. Samples taken during the summer of 1986 have revealed a low density of zooplankton (c. 1 individual/l, June 1986) and some high density of benthos (c. 1000 individuals/m^2, June 1986).

Extensive fishing trips to the pond during summer 1985 and 1986 have shown no sign of predatory fish in the lake which is dominated almost exclusively by three-spined sticklebacks; apart from sticklebacks, only one individual of minnow (Phoxinus phoxinus) has been caught from the Pond. Because the Pond is situated close to Loch Lomond, its sticklebacks are likely to be subjects to predation by avian predators similar to those of Loch Lomond. However, the increased vegetation cover in Balmaha Pond may act to reduce the intensity of predation level from avian predators.

River Kelvin

The River Kelvin is a fast flowing urban river. Fish species, such as eel, brown trout, stone loach, minnows, ten-spined sticklebacks, and three-spined sticklebacks exist in the river (Clyde River Purification Board survey, unpub. data). Many predatory birds are known to inhabit the area. These include kingfisher (Alcedo atthis), heron, black headed gull, common gull and arctic tern (Gibson 1981).

For logistic reasons, most of the laboratory tests were carried out at different times of the year using fish population (River Kelvin) different from those of field tests (Loch Lomond and Balmaha Pond fish). However, the behaviour of the fish was comparable and this was thought not to influence the results.

2.2 The experimental fish

All the experimental fish were hand-netted from shallow waters (up to 1.5 m deep), usually in vegetation. Fish were transferred to the laboratory in opaque plastic containers and housed in large communal tanks (at a density of up to 30 fish/200 l water), usually under ambient temperature and dark-light cycle. The tanks were filled with natural water or tap water with added tap-water conditioner (a commercial treatment to prevent chemical shocks to the fish). The tanks bottoms had layers of gravel and some weed cover. To reduce the concentration of ammonia, partial changes of the water were carried out every 1-2 weeks and a constant aeration system was provided. Treatments against diseases (especially white spot, Ichthyophthirius multifiliis) were applied when necessary. Great care was taken to minimize disturbing the captive fish.

All fish used were non-breeding adults, fish showing breeding activities (males defending territories or females with eggs) were not tested. Thus, female sticklebacks were used more frequently than males. Only fish which appeared perfectly healthy were tested. Fish infected with tape worms (Schistocephalus solidus), which could be recognized from their swollen abdomens, were not used as infection modifies host behaviour and causes them to take more risk against predators than uninfected fish (Milinski 1985, Giles 1987 a).

In most of the tests described below, sticklebacks were tested individually in order to provide uniform test situations and to avoid the effect of competition among fish. Sticklebacks may occur naturally in schools and thus the behaviour of solitary fish may differ from those in the schools. It was thought that the advantages of using solitary fish overweigh the disadvantages. However, in order to keep the duration of some field tests at minimum (to minimize the variability in the environmental conditions), sticklebacks were tested in small groups (3 fish at a time); the size of these groups still minimized the interaction between fish since the tests were run in large enclosures (at least 1 m in diameter).

When necessary, sticklebacks were killed by Benzocain anaesthesia (150 mg/l) and preserved in 70% alcohol. Sample sizes were kept at the levels which provide reliable estimates of fish behaviour, while minimizing the number of fish killed.

For practical reasons, laboratory predation-tests were carried out using predator (model kingfisher) different from those of field predation-tests (live trout), the responses of sticklebacks to both predators were comparable and this was thought not to influence the results.

2.3 Stomach contents analysis and morphological studies

Analysis of stomach contents began with dissecting the fish and separating its stomach out on a glass slide with few drops of water. The stomach was then bisected longitudinally and the contents were removed into a cavity glass slide under a binocular microscope.

All morphological analysis of the fish and identification of the invertebrates (zooplankton and benthos) were carried out under a suitable magnification of a binocular microscope. Higher magnification was also used to inspect stomach contents for microscopic organisms. Identification of the invertebrates was made according to the identification keys produced by Freshwater Biology Association.

2.4 Data recording and statistical analysis

The event durations in the laboratory tests were usually made on a microcomputer which was programmed to give the time to the nearest 0.1 sec. The data were analysed by using Minitab package when appropriate. The distribution of the data was usually far from normal and in many cases various transformation methods failed to normalize it. Therefore, non-parametric tests were used in most of the analyses and the sample median (with range) was presented as an estimate of the

population, since it is less affected by the outlying distribution than the mean. However, in few cases, where the required assumptions were met, parameteric tests were used. The level of significance was always set at 0.05. In some of the figures presented in this thesis, the ranges were presented in separate tables for the ease of presentation.

CHAPTER 3

FOOD PROFITABILITY AND FEEDING EFFICIENCY OF STICKLEBACKS

3.1 INTRODUCTION

3.1.1 Measuring feeding profitability

The benefits associated with a particular manner of feeding are usually expressed as the profitability of that feeding, which is the total energetic contents of the prey divided by the total cost of acquiring this energy. The total energy derived from food is relatively easy to determine; ecologists usually measure this energy in terms of the dry weight of the food or the actual energetic value. The energetic value of the food is the more realistic measure of the benefit especially when dealing with more than one type of food, simply because different prey types with the same dry weight may have different energetic contents.

The total energy of the food eaten by an animal is either used for metabolism, growth, and any reproductive products or is lost in the faeces and excretory products (Elliott 1976). The latter loss of energy could reach 20% of the total energy obtained (Winberg 1956), and therefore should be taken into account when considering the actual energy gain of consuming the prey.

Measurement of the foraging costs has been made in various ways:

a. By measuring the energetic cost of handling the prey (Stein et al. 1984).
b. By measuring the effect on the growth rate of foragers (Griffiths 1980 a).
c. By estimating the time spent finding and dealing with the food items (Werner and Hall 1974, Kislalioglu and Gibson 1976 a, Gibson 1980). This latter method is, in most circumstances, realistic and relatively quick and easy to achieve.

Deriving predictions from Optimal Foraging Theory is complicated by the fact that the costs (and the benefits) of a particular foraging strategy may depend on many factors such as the overall manner of feeding, the size of the fish, the size of the prey, and the complexity of the habitat. Generally, the times spent searching, pursuing, fixating, and handling the prey are the major components of the cost, and some authors have included the cost of digestion. However, since animals vary in their foraging strategies (Curio 1976), the relative importance of the cost components varies accordingly (Griffiths 1980 b). For example, planktivorous filter feeders spend small amounts of time pursuing and handling their prey, but they have to make contact with their food. This indicates the importance, for such feeders, of which might be called search time rather than handling time. Conversely, search time for animals following a sit-and-wait feeding strategy is not as important as pursuing and handling time.

Accordingly, for sticklebacks which search actively for food and encounter a wide variety of prey types and sizes, searching, fixating and handling times all are important when determining the energetic costs of foraging. Search time is largely related to prey density and has an important role in determining the cost of feeding on natural populations of the prey. However, when prey items are made readily available to animals, search time can be neglected, and fixating and handling times become the major costs associated with feeding.

3.1.2 Variation in feeding costs and the feeding efficiency

The cost of time spent feeding on a given food type is a decreasing function of the level of hunger of fish (due to increasing rate of attacks with hunger, Ware 1972) and the size of fish (Unger and Lewis 1983, Colgan *et al.* 1986) but it is an increasing function of prey size (Stein *et al.* 1984). Moreover, searching for food is likely to take longer in complex habitats, so the efficiency of a forager may be decreased with increasing habitat complexity (Crowder and Cooper 1982).

The experience of a forager with food may increase the efficiency with which prey are captured. This comes about by decreasing the cost associated with the familiar prey types (Dill 1983). Thus, observations on three-spined sticklebacks have indicated that larger, older fish are more able to select an optimal diet than smaller fish (Chapter 4). Studies on the improvement in efficiency with experience have often involoved investigation of the changes in attack success, usually defined as the percentage of attacks that result in prey capture (eg. McComas and Drenner 1983, Colgan *et al.* 1986). According to such parameter of feeding efficiency, it is difficult to define where the improvement in feeding efficiency lies. It may be due to decreasing rejection rate of captured prey or to increasing ability of the predators to detect, catch or manipulate the prey.

Fish from different localities of different available invertebrate fauna may differ in their feeding efficiency when feeding on a particular food type. Benthic sticklebacks (which inhabit areas of thick vegetation and feed on benthos) are more successful at capturing organisms that inhabit the lake bed than limnetic sticklebacks (which inhabit open water areas and feed on zooplankton); these in turn are

more efficient at feeding on zooplankton (Bentzen and McPhail 1984). These differences in feeding efficiency between limnetic and benthic sticklebacks correlate with the morphology of the feeding apparatus. Limnetic form of stickleback possesses a narrower mouth and higher, closer, and longer gillrakers whereas benthic form possesses the opposite properties. Feeding efficiency of fish in general and of sticklebacks in particular has so far been measured by means of total number of prey captures (eg. Bentzen and McPhail 1984) without reference to the actual energetic value of the prey. This measure is unsatisfactory since a fish consuming many prey items of relatively low energetic values may obtain the same benefit as a fish consuming fewer prey of higher energetic values, yet the number of prey consumed is different.

3.1.3 Aims

The aims of the experiments described in this chapter are:

1. To measure, in a simple laboratory experiment, fixation and handling times spent by various sizes of sticklebacks when feeding on different prey types and sizes, and to measure the energetic contents of such prey in order to determine their profitabilities.

2. As a subsidiary aim, to investigate the effect of number of prey eaten on handling time and to examine the interactive effect of fish and prey sizes and types in terms of fixation and handling times.

3. To investigate in detail the effect of experience on various components of foraging (viz. feed latency, fixation and handling times and number of grasps required to swallow a prey item).

4. To investigate the foraging efficiency, in terms of net energetic rewards, in sticklebacks from two different local populations feeding on a range of prey types in more complex environments (simulated field conditions).

3.2 LABORATORY STUDIES OF PREY PROFITABILITY

3.2.1 Material and Methods

a. Determination of fixation and handling times

The fish

The fish used in this test were caught in the River Kelvin (Glasgow) during November 1984. They were chosen to represent three size classes, small (34-36 mm, S.L), medium (4^{0}-4$\frac{1}{2}$ mm, S.L.) and large (47-50 mm, S.L.). A sample size of 6 fish from each group was tested; this sample size was found to be sufficient to stabilize estimates of handling time. The fish were kept individually in white plastic basins (5 l each) for three weeks prior to the experiment and fed daily on alternate meals of Chironomid larvae, Tubifex worms, Daphnia sp., and Asellus aquaticus. Thus, the fish were assumed to

have experienced all these prey types in the laboratory in equal proportions. Water temparature varied between 11 and 14°C.

The prey

Four different prey types were used in this study; Chironomid larvae, Tubifex, Daphnia sp., and Asellus aquaticus. These prey were chosen because they are known to be the major food types of sticklebacks and are available in the habitat where the fish were caugnt. Also they provide a wide range of different physical stimuli (such as colour, movement, shape, and size) and were therefore used in studies of the stumuli governing food selection of sticklebacks (Chapter 4).

Chironomid larvae, Daphnia and Tubifex were obtained commercially, while Asellus were collected from the River Kelvin. From each prey type, three size classes (small, medium, and large) were tested separately. Prey measurements (length, from the top of the head to the end of the abdomen, and thickness, dorso-ventral distance) were taken to the nearest 0.05 mm and these are presented in Table 3.1.

The test tank

The tests were conducted in a glass tank (32x16x25 cm) divided into two equal compartments (a resting compartment and a test compartment) by an opaque partition with a sliding door operated from outside. The tank was filled with water till 7 cm deep and surrounded with white paper to minimize the disturbance from outside. The observations were made through a sheltered slit.

Test protocol

At the start of each test, 10 individuals of a given prey category were introduced into the feeding compartment. A stickleback was transferred gently into the start compartment and allowed to settle down for 5-10 min (5 min were enough and 10 min made no difference) then the door was opened, giving the fish access to the food. The fish usually entered the test compartment within 1-2 min and immediately reacted to the prey; thus search time was negligible.

Fixation time was recorded as the period between the initial approach of the fish and the first contact with the prey. Fixation time for Daphnia was too short to be measured and thus was not recorded. For the other prey types, the total time spent fixating prey items during the whole test (5 min, see below) was used in the analysis because fixation time for a single prey individual was too short for accurate measurement. Handling time was defined as the time that elapsed between first contact with the prey and swallowing it. The exact time of swallowing a prey item was regarded as the end of a series of rapid jaw movements after grasping the prey, which in some cases followed by abduction of the jaw as a sign of swallowing the prey. If the prey were all consumed, another group of 10 individuals was then introduced by a screened pipette into the feeding compartment.

The test was terminated 5 min after the first reaction of the fish to the prey, by which time the feeding rate had often noticeably declined. The fish was then fed to satiation in preparation for another test 24 h later. Each fish was tested once with each type and size of prey in a matched design. Tests with different prey types and sizes were alternated so that the fish never got the same prey

type or size on any two successive days. In this way any effect of experience with a particular prey was minimized. All the fish used were starved for 24 h (to evacuate their stomachs, Beukema 1968) because satiation can reduce success rate and increase handling time (Werner 1974).

Table 3.1: Mean length and thickness (mm ± S.E.) of various prey types and sizes used in determining fixation and handling times.

PREY TYPE / SIZE	MEAN LENGTH	MEAN THICKNESS
Chironomid larvae:		
Small	6.7 ± 0.11	0.45 ± 0.02
Medium	10.7 ± 0.90	0.79 ± 0.01
Large	15.5 ± 0.16	1.16 ± 0.02
Tubifex:		
Small	10.0 ± 1.34	0.42 ± 0.01
Medium	20.0 ± 1.70	0.60 ± 0.02
Large	28.0 ± 1.22	0.89 ± 0.02
Daphnia:		
Small	1.5 ± 0.03	0.85 ± 0.02
Medium	2.1 ± 0.04	1.17 ± 0.02
Large	3.0 ± 0.04	1.81 ± 0.02
Asellus:		
Small	3.9 ± 0.07	0.98 ± 0.03
Medium	6.0 ± 0.12	1.53 ± 0.03
Large	8.5 ± 0.18	2.25 ± 0.05

b. Determination of energetic content

A known number of items (50-500 depending on prey size) with three replicates of each prey type was placed in a 60°C oven until a constant dry weight was obtained. The dry weight (to the nearest 0.05 mg) for the whole sample and hence for a single item was obtained. Samples were ground and at least three pellets (7-18 mg each, the optimal size according to Paine 1971) were prepared from each, weighed, and the number of intact items in each pellet was calculated. The number of calories per pellet was determined using a micro-bomb calorimeter (Newham Electronic, model AH9) and hence the number of calories per one mg dry weight and per one item was calculated. The energetic content was then expressed in terms of joules (1 calorie = 4.184 joules).

This method (Phillipson 1964, see also Paine 1971 for comments) is based on firing the sample and recording the total heat output in terms of arbitrary units. These units can then be converted to calories by calculating the weight of benzoic acid which produces the same number of heat units. This was obtained from a simultaneously prepared calibration graph between weight of benzoic acid and the number of units produced in the same machine. Since 1 mg benzoic acid generates a known number of calories (6.324 cal), the total calories in the sample can be easily calculated. The regression equation for the calibration graph was:

$$Y = 0.2231X + 0.4316$$

where:

Y = Weight of equivalent benzoic acid.
X = Number of units recorded.

X and Y were highly correlated (product moment correlation coefficient = 0.98, n = 10, P <0.001).

All calculations were based on ash-free dry weight of the samples. Acid production (nitric and sulfuric) correction was not made since this source of error is minor (Paine 1971).

c. Profitability calculations

Handling and fixation times for the first prey eaten were used in all calculations of profitability, because in some cases the fish were satiated by the first prey eaten and took more variable times to handle the second (see below). Prey profitability was calculated for a given size of fish in two ways:

a. By dividing the number of joules of a given prey category by mean handling time (sec).
b. By dividing the number of joules of a given prey category by mean total handling and fixation times (sec).

3.2.2 Data analysis

Handling time and number of prey eaten

To test for the effect of number of prey eaten on handling time, the time taken by fish to handle the first prey was subtracted from that of the second prey. A one-sample t-test (Bailey 1981) was then used

to test whether the mean differences is significantly different from zero.

Effect of prey size, fish size and prey type on fixation and handling times

A Friedmann two-way ANOVA by ranks was used to test the effect of prey size, for a given fish size, on fixation and handling times. A Kruskal-Wallis one-way ANOVA by ranks was used to test the effect of fish size, for a given prey category, on fixation and handling times. To test the effect of prey type on fixation and handling times, the data of the three sizes of a given prey type were averaged for each fish, and a Friedmann test was then used to test the differences.

3.2.3 Results

a. Fixation and handling times

Effect of prey ingestion number on handling time

The mean handling times of the first and the second prey eaten are presented in Table 3.2. Handling time increased significantly from the first to the second prey in all sizes of prey except for small Chironomid larvae, small Tubifex and small & medium Daphnia. Because of the way fixation time was calculated (for the whole 5 min of the test, see above), the effect of prey ingestion number on fixation time was not considered.

Effect of prey size on fixation and handling times

The median times spent by various sizes of fish in fixating and handling various sizes of prey are presented in Fig. 3.1 and Fig. 3.2 respectively. All size classes of fish increased fixation time as the prey size increased. This increase was not significant in the case of medium size of Chironomid larvae & Tubifex and small & medium sizes of Asellus (fixation time for Daphnia was not recorded, see above). Similarly, all size classes of fish increased the time spent handling a given prey category as the prey size increased. This increase was significant in the case of Chironomid larvae and Tubifex, but not in the case of Daphnia. Size classes of Asellus were not compared because large ones were never eaten and medium ones were only taken by medium and large fish.

Effect of fish size on fixation and handling times

The median times spent by various size classes of fish in fixating and handling a given size of prey are presented in Fig. 3.3 and Fig. 3.4 respectively (rearranged from Fig. 3.1 and Fig. 3.2 respectively to highlight the effect of fish size). Fixation time decreased with increasing fish size, but this decrease was significant only in the case of small Chironomid larvae and small Asellus. Handling time also decreased as fish size increased. This decrease was significant in the case of large Chironomid larvae, medium & large Daphnia, and small Asellus. Again medium and large Asellus were not compared for the same reason mentioned above.

Effect of prey type on fixation and handling times

The median times spent by the fish in fixating and handling each prey type are presented in Tables 3.5 and 3.6 respectively. Fixation time was longer for Asellus than for either Tubifex or Chironomid larvae. Handling time was lower in the case of Daphnia than in the case of Tubifex and Chironomid larvae.

Table 3.2: Mean handling times (sec ± S.E.) of the first and the second prey of various sizes and types eaten by sticklebacks.
(* = P <0.05, ** = P <0.01, NS= not significant; T-test)

PREY TYPE/SIZE	MEAN HANDLING TIME		
	FIRST PREY	SECOND PREY	
Chironomid larvae:			
Small	2.05 ± 0.24	2.35 ± 0.23	NS
Medium	2.95 ± 0.34	4.26 ± 0.68	*
Large	13.51 ± 3.53	18.20 ± 3.40	**
Tubifex:			
Small	2.85 ± 0.34	3.18 ± 0.40	NS
Medium	4.61 ± 0.22	5.38 ± 0.35	*
Large	5.36 ± 0.42	7.95 ± 0.58	**
Daphnia:			
Small	1.24 ± 0.10	1.26 ± 0.08	NS
Medium	1.34 ± 0.16	1.65 ± 0.15	NS
Large	1.88 ± 0.20	2.31 ± 0.24	*
Asellus:			
Small	3.87 ± 0.66	4.72 ± 0.65	*
Medium	7.20 ± 1.01	17.70 ± 3.89	*
Large	-	-	

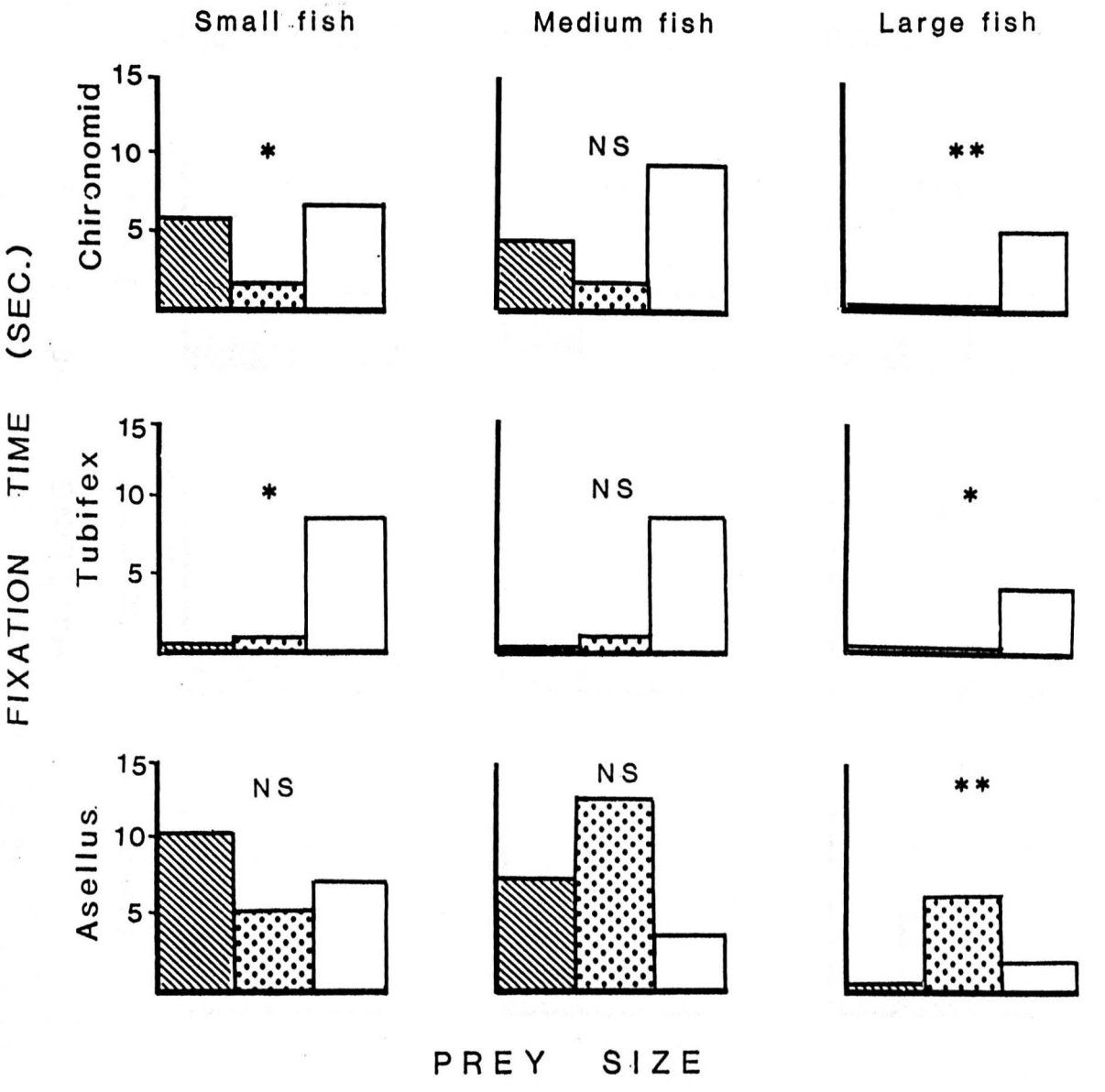

Fig. 3.1: Median times (sec) spent by various sizes of fish in fixating small (lined columns), medium (dotted columns), and large-sized prey (blank columns) of various types. The range is given in Table 3.3.
(* = P <0.05, ** = P <0.01, NS = not significant, Friedmann-test)

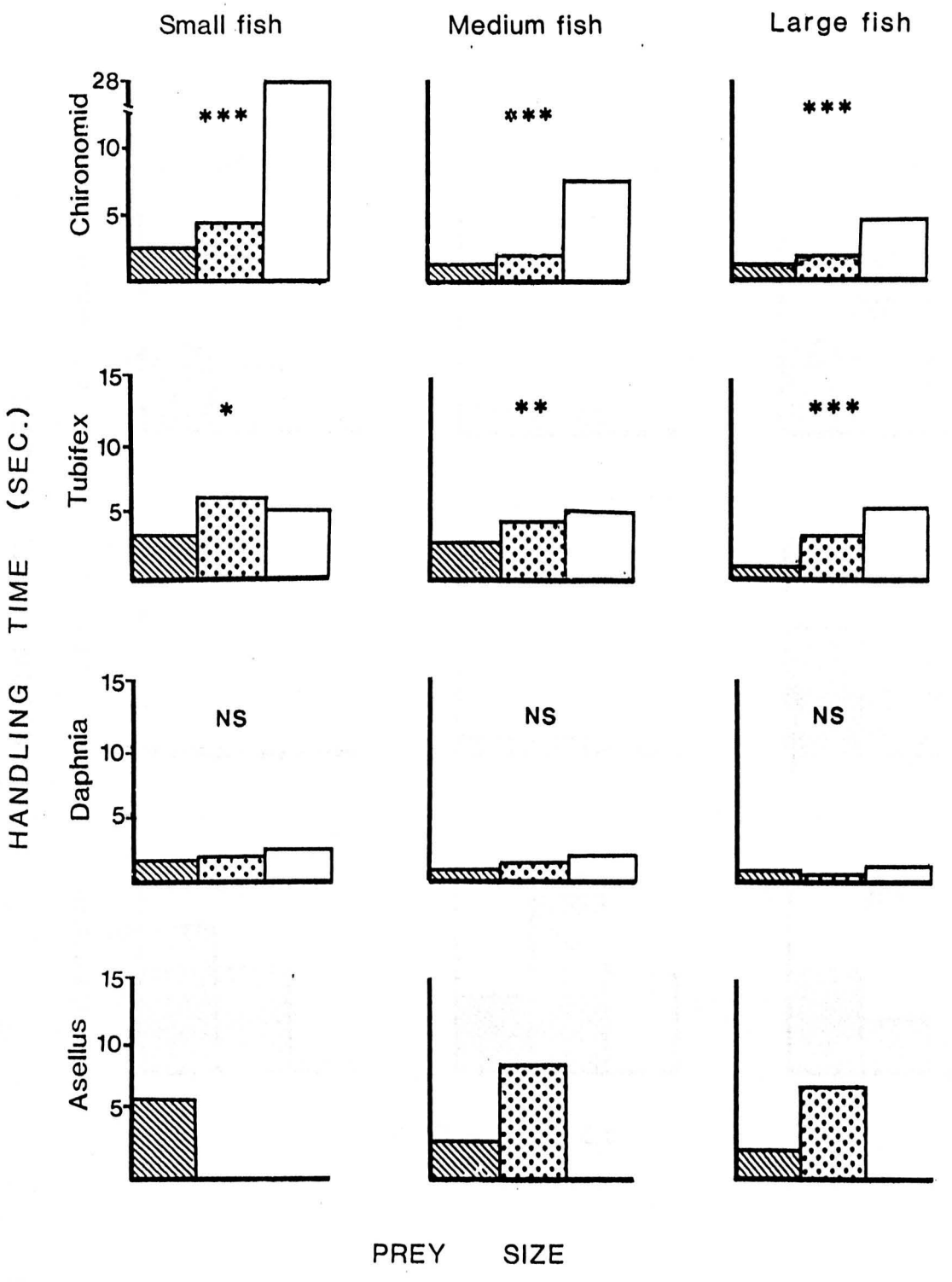

Fig. 3.2: Median times (sec) spent by various sizes of fish in handling small (lined columns), medium (dotted columns), and large-sized prey (blank columns) of various types. The range is given in Table 3.4. (* = P <0.05, ** = P <0.01, *** = P <0.001, NS = not significant, Friedmann-test)

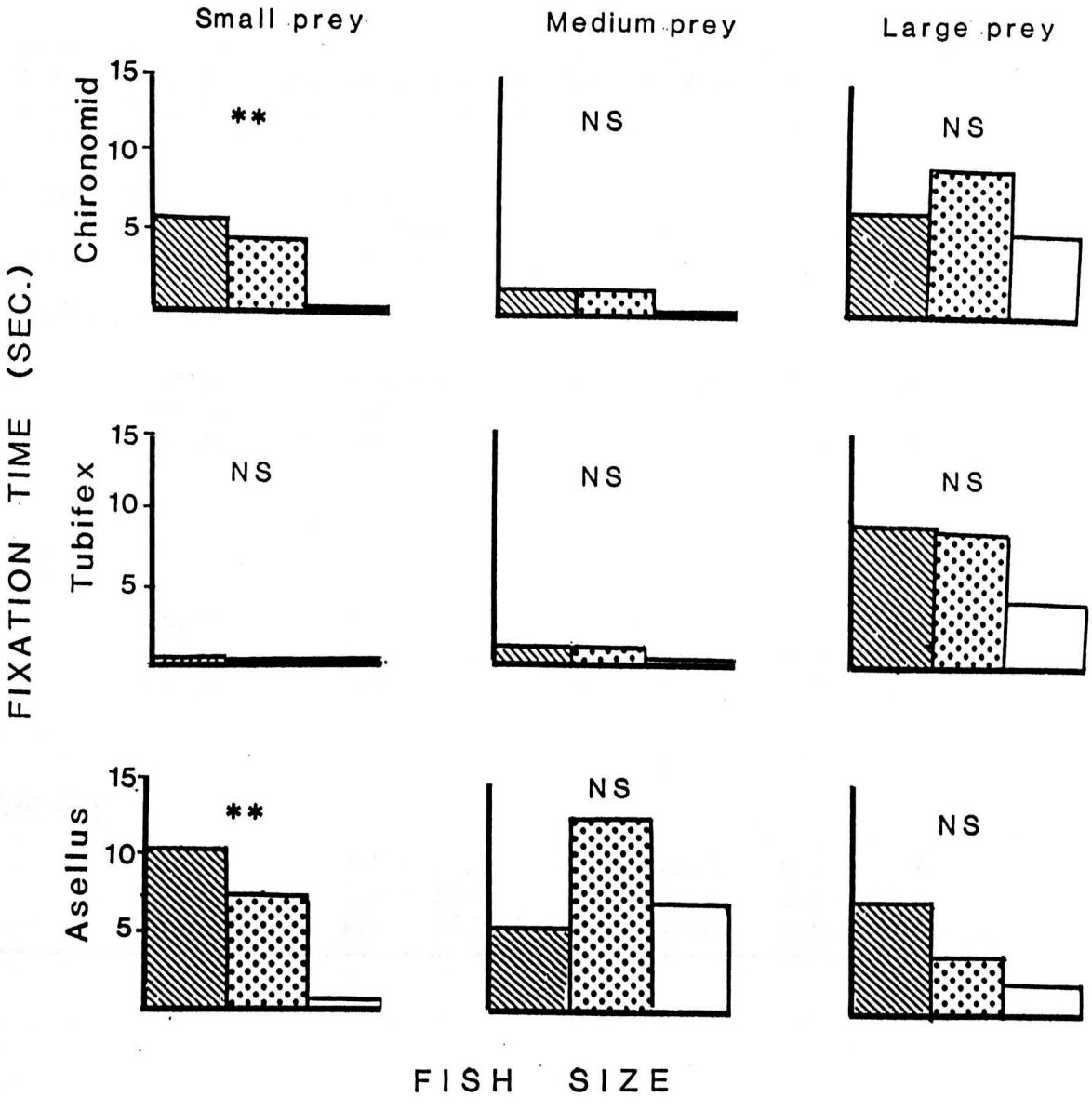

Fig. 3.3: Median times (sec) spent by small (lined columns), medium (dotted columns), and large fish (blank columns) in fixating various sizes and types of prey (rearranged from Fig. 3.1 to highlight fish-size effect). The range is given in Table 3.3.
(** = P <0.01, NS = not significant, Kruskal-Wallis-test)

Table 3.3: Range of time spent by various sizes of fish in fixating small, medium and large-sized prey of various types (complementary to Fig. 3.1 and Fig. 3.3).

PREY TYPE / SIZE	FISH SIZE		
	SMALL	MEDIUM	LARGE
Chironomid larvae:			
Small	1.5 - 11.9	4.4 - 10.8	0.3 - 0.6
Medium	1.0 - 5.5	0.4 - 5.0	0.2 - 0.9
Large	5.4 - 17.3	4.4 - 28.0	1.2 - 8.0
Tubifex:			
Small	0.3 - 3.3	0.4 - 1.2	0.3 - 0.8
Medium	1.1 - 10.3	0.9 - 10.0	0.5 - 40.0
Large	4.9 - 12.0	2.0 - 13.6	2.0 - 22.1
Asellus:			
Small	3.3 - 29.3	1.5 - 21.2	0.2 - 1.6
Medium	1.9 - 10.0	5.8 - 40.4	3.0 - 14.1
Large	1.0 - 16.6	1.5 - 11.6	1.5 - 6.5

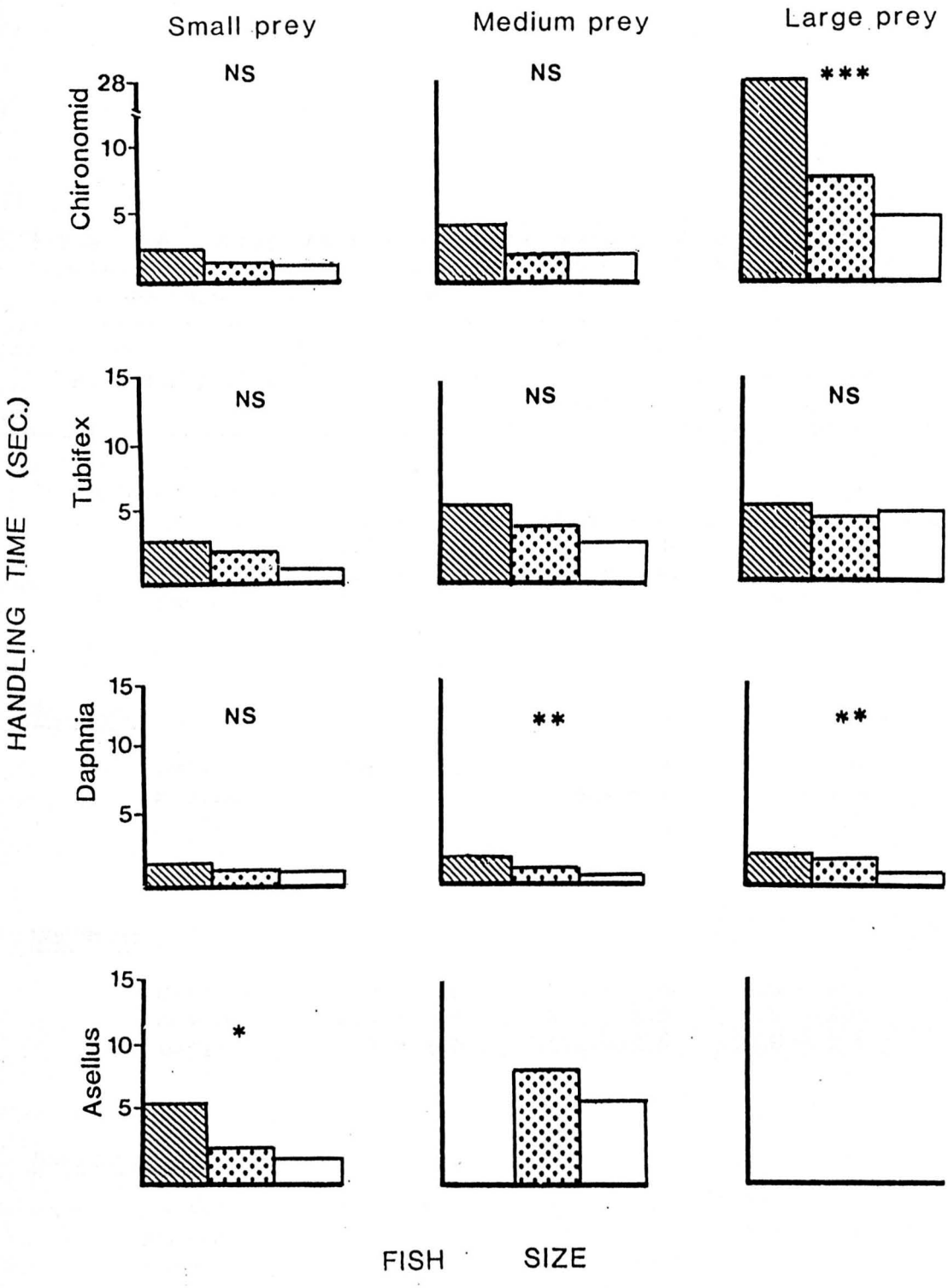

Fig. 3.4: Median times (sec) spent by small (lined columns), medium (dotted columns), and large fish (blank columns) in handling various sizes and types of prey (rearranged from Fig. 3.2 to highlight fish-size effect). The range is given in Table 3.4.
(* = P <0.05, ** = P <0.01, NS = not significant, Kruskal-Wallis-test)

Table 3.4: Range of time spent by various sizes of fish in handling small, medium and large-sized prey of various types (complementary to Fig. 3.2 and Fig. 3.4).

PREY TYPE / SIZE	FISH SIZE		
	SMALL	MEDIUM	LARGE
Chironomid larvae:			
Small	1.4 - 5.0	1.4 - 2.5	1.3 - 2.8
Medium	1.1 - 5.2	1.5 - 3.7	1.3 - 3.4
Large	10.9 - 60.1	5.5 - 8.7	2.3 - 7.6
Tubifex:			
Small	2.1 - 4.3	1.7 - 5.3	1.2 - 3.0
Medium	3.8 - 8.6	3.5 - 5.4	1.8 - 4.3
Large	5.0 - 6.9	3.0 - 7.2	3.3 - 6.2
Daphnia:			
Small	0.9 - 1.8	0.9 - 1.5	0.8 - 1.5
Medium	1.0 - 3.0	0.8 - 2.2	0.7 - 1.4
Large	1.8 - 4.5	1.2 - 2.5	0.8 - 1.6
Asellus:			
Small	4.3 - 8.2	2.2 - 4.3	1.8 - 3.1
Medium	-	4.8 - 10.1	4.6 - 9.8
Large	-	-	-

Table 3.5: Median times (sec) spent by various size classes of fish in fixating Chironomid larvae, Tubifex and Asellus. The range is given between brackets.

FISH SIZE	PREY TYPE		
	Chironomid	Tubifex	Asellus
SMALL	5.71 (3.8-12.6)	3.01 (1.9-6.2)	6.00 (3.6-22.1)
MEDIUM	5.35 (4.4-10.8)	3.75 (0.7-8.4)	9.20 (2.7-28.7)
LARGE	1.60 (0.4-2.6)	1.95 (0.7-7.3)	3.15 (1.8-6.2)

$X^2r = 98.3$, n = 18, k = 3, P <0.001; Friedmann-test

Table 3.6: Median times (sec) spent by various size classes of fish in handling Chironomid larvae, Tubifex and Daphnia. The range is given between brackets.

FISH SIZE	PREY TYPE		
	Chironomid	Tubifex	Daphnia
SMALL	10.1 (5.6-22.3)	4.85 (3.6-6.8)	2.11 (1.4-2.4)
MEDIUM	3.70 (3.4-4.2)	3.80 (2.9-5.9)	1.48 (0.8-1.9)
LARGE	3.03 (2.3-4.0)	3.25 (2.4-4.3)	1.01 (0.8-1.2)

$X^2r = 27.9$, n = 18, k = 3, P <0.05; Friedmann-test

b. Energetic content

The number of joules contained in 1 mg dry weight and in one prey item of various prey types and sizes is given in Table 3.7. Energetic contents clearly increased with increase in prey size. Chironomid larvae are energetically the richest food type (with higher number of joules per mg dry weight), followed by Tubifex and Daphnia, while Asellus are the lowest.

c. Profitability values

The two ways of calculating the profitability (by dividing the total number of joules by mean handling time and by mean total handling and fixation times) gave similar results because fixation time for one single prey item was short in all cases. Thus, because fixation time was not recorded for Daphnia and in order to standardize the calculations between all prey types, the profitability based on the cost of handling time alone was used and the data are presented in Table 3.8. Considering the effect of prey size, medium Chironomid larvae were more profitable than the large and the small Chironomid larvae. Profitability values for Tubifex and Daphnia increased with prey size, while it decreased from small to medium-sized Asellus (for medium and large fish). For a given prey size, the profitability value usually increased as fish size increased.

Table 3.7: Mean number of joules (± S.E.) contained in 1 mg dry weight, mean dry weight per item (mg ± S.E.) and number of joules contained in one item of various prey sizes and types used in determining the profitability values.

PREY TYPE	MEAN NO. OF JOULES PER mg DRY WEIGHT	MEAN DRY WEIGHT PER ITEM			JOULES PER ITEM		
		SMALL	MEDIUM	LARGE	SMALL	MEDIUM	LARGE
Chironomid	21.50 ± 0.08	0.36 ± 0.006	0.86 ± 0.020	1.76 ± 0.110	7.74	18.49	37.84
Tubifex	20.44 ± 0.07	0.31 ± 0.040	0.80 ± 0.050	1.48 ± 0.030	6.33	16.35	30.25
Daphnia	17.57 ± 0.11	0.033 ± 0.003	0.055 ± 0.004	0.163 ± 0.008	0.58	0.96	2.86
Asellus	15.48 ± 0.07	1.00 ± 0.040	2.10 ± 0.080	3.50 ± 0.270	15.48	32.51	54.18

Table 3.8: Profitability values (joules/sec) of various prey types and sizes for various sizes of sticklebacks.

PREY TYPE / SIZE	FISH SIZE		
	SMALL	MEDIUM	LARGE
Chironomid larvae:			
Small	2.83	3.87	3.91
Medium	4.35	7.25	8.45
Large	1.34	5.10	7.93
Tubifex:			
Small	1.36	2.30	3.51
Medium	2.52	3.47	4.42
Large	5.54	5.78	5.56
Daphnia:			
Small	0.40	0.58	0.53
Medium	0.54	0.73	1.07
Large	1.15	1.49	2.31
Asellus:			
Small	2.50	5.15	7.00
Medium	-	4.20	4.33
Large	-	-	-

3.2.4 Discussion

a. Fixation and handling times

The experiments have shown that handling time is an increasing function of both prey capture number and prey size, and a decreasing function of fish size. Handling time for the second prey eaten is longer than that for the first, especially for larger sizes of prey; for small sized prey, handling time has not been increased significantly with increase in prey number. This increase in handling time seems to be related to the amount of food in the stomach since this would increase handling time (Werner 1974, Kislalioglu and Gibson 1976 a); after eating the first prey the fish become more satiated and consequently handling time for the next prey becomes longer. Accordingly, since larger prey contribute more to the amount of food in the stomach than smaller prey, one could expect handling time for a prey eaten after large prey item to be longer than if it is eaten after a small one. This difference in handling time raises a problem when extrapolating the profitability value of a prey at one level of satiation to another.

Handling time also increases as prey size increases. This increase can be attributed to the increase in the ratio of prey size to fish size which results in decreasing the ability of the fish to handle the prey. The same result has also been shown in other laboratory studies (Kislalioglu and Gibson 1976 a) and this may have implications for the behaviour of fish in the field (Kislalioglu and Gibson 1975, Unger and Lewis 1983) where larger fish tend to include larger prey items in their diet. However, the degree of reduction in handling times with

increase in fish size is more obvious (i.e. the difference is significant) in the case of larger prey items (large Chironomid larvae, medium and large Daphnia, and small Asellus, Fig. 3.4) which have prey thickness of 0.98 mm or more (Table 3.1). Conversely, in the case of the remaining prey items used, where the reduction lacks significance, the prey have thicknesses of less than 0.98 mm. This indicates that fish of various sizes have a threshold prey thickness above which handling time will be prolonged.

The result also shows that fixation time increases with prey size and decreases with fish size. Thus, larger prey are fixated and handled longer than smaller prey and larger fish spend shorter time fixating and handling a prey than smaller fish. Again, the prey types which require longer handling also receive longer fixation. Fixation time therefore may indicate the fish's reluctance or fear of the prey, with smaller fish fearing prey of a given size more than larger fish, and fish of a given size fearing larger prey more than smaller prey.

b. Prey profitability

The definition of prey profitability as energetic contents/handling time does not consider the energetic cost of handling the experimental prey. The relative energetic expenditure in handling two different prey types may differ because they may be handled in different ways with different relative energetic costs. O'Brien (1979) has reported that small lake trout carefully position themselves in front of an evasive copepod and then swim towards it and simultaneously open their mouth in an attempt to suck it in. In contrast, fish casually swim

towards and hesitate in front of a non-evasive daphnid before sucking it into the mouth. The energetic expenditure by the fish to catch these two contrasting prey may differ markedly even if the handling times are equal. However, the four prey types offered to the sticklebacks in my study are all non-evasive and the differences in energetic expenditure are assumed to be low. Thus, the relative profitabilities of such prey should not be influenced by the energetic costs of handling.

The Profitability value of a given prey increases with fish size. Since the energetic content of a given prey is constant; the increased profitability with fish size is undoubtedly due to the shorter time spent by larger fish to handle that prey.

Profitability value increases with prey size in the case of Tubifex and Daphnia but not in the case of Chironomid larvae and Asellus; larger Chironomid larvae and medium Asellus are less profitable than medium Chironomid larvae and small Asellus respectively. Given that the energetic contents increase with prey size (Table 3.7), this decrease in the profitability value is due to the longer time spent by fish of various sizes in handling large Chironomid larvae and medium Asellus compared to medium Chironomid larvae and small Asellus respectively (Fig. 3.2).

The profitability values obtained in this study have been determined by using the cost paid by the fish to handle the first item of a given prey type. Thus, if the successive prey items are to be considered, then the profitability value may become lower due to prolonging handling time (see above).

3.3 EXPERIENCE AND FEEDING EFFICIENCY IN STICKLEBACKS

3.3.1 Material and Methods

The fish

Two groups of fish from the River Kelvin (Glasgow) were used in these experiments. One group was hatched during July 1986 and reared in the laboratory at a temperature of 10-12^{o}C and a light regime of 12 h light X 12 h dark. During the first few days they were fed on liquifry (a commercial food for newly born fish); they were then fed on live brine shrimp nauplii for two weeks before they were gradually transferred to an artificial diet (frozen brine shrimp). In March of the following year (1987) another group of fish, with similar size distribution, was caught in the River Kelvin; the two groups had similar standard length, i.e. 31-33 mm. These wild caught fish were also fed on frozen brine shrimp for 4 weeks before testing them.

The test tank

Tests were carried out in a transparent perspex tank (19x12x25 cm) divided into two sections by a transparent partition with a sliding door (4x4 cm) operated from outside by a thin white thread. The two sections were called the start compartment (10x12x25 cm) and feeding compartment (9x12x25 cm). The water level in the tank was 7 cm and the walls and bottom were covered with white paper. The observations were made through a shielded slit.

The prey

The fish were tested on 3 different types of live prey; Chironomid larvae, Asellus aquaticus, and Daphnia sp., which have mean lengths (± S.E.) of 7 ± 0.10 mm, 3 ± 0.06 mm, and 2.5 ± 0.06 mm respectively. These prey types were chosen because they are known to be a natural food of these fish.

Test protocol

The prey were added in groups of 4 individuals of a given prey type. Each fish (deprived of food for 24 h) was introduced singly into the start section of the test tank and left there for 5 min to settle down; then a door was opened giving the fish access to the feeding compartment. The data recorded included:

a. Feed latency = the time elapsing between entering the feeding area and beginning of a successful strike at the prey.
b. Fixation time = the time spent by fish observing the prey before grasping and eating it.
c. Handling time = the time spent between first contact with the prey and swallowing it.
d. Total grasps = the number of times the prey was taken into the mouth of the fish before being eaten.

Fifteen laboratory-reared fish and 15 wild-caught fish were tested on a given prey type, and tests were alternated between fish of the two groups. The test was terminated after one prey item had been consumed. Following this test the fish were fed for 6 days on alternate daily meals of the three prey types (i.e. the fish had two meals of each prey type) then retested in the same way for the same variables.

3.3.2 Data analysis

A Mann-Whitney U-test was used to test the differences between the laboratory-reared and wild-caught fish in the time spent in various feeding acts as well as the difference in the total number of grasps before eating the prey. This test (U-test) was also used to test the differences between the first and the second tests in various feeding parameters under consideration.

3.3.3 Results

Behaviour of laboratory-reared and wild-caught fish in the first test

When feeding on Chironomid larvae and Daphnia, naive, laboratory-reared sticklebacks took significantly longer than wild-caught fish to strike successfully at the prey. No significant difference in feed latency was found between the two groups when feeding on Asellus; the latency was long in both cases (Fig. 3.5 a). Fixation times (Fig. 3.5 b) and handling times (Fig. 3.5 c) were longer for laboratory-reared sticklebacks for all prey types. Laboratory-reared sticklebacks feeding on Daphnia (but not on the other 2 species) took more grasps to swallow the prey than wild-caught fish (Fig. 3.5 d).

Comparison between the first and the second tests

As Fig. 3.5 shows, feed latency in the laboratory-reared fish declined from the first to the second test for all prey types (not significant in the case of Asellus). In wild-caught fish feed latency decreased for Asellus but not for the other prey types. Fixation and handling times decreased for all prey types in laboratory-reared fish and in wild-caught fish for Daphnia as well as for Asellus (handling time). The number of grasps required before eating the prey decreased in the case of laboratory-reared fish feeding on Daphnia.

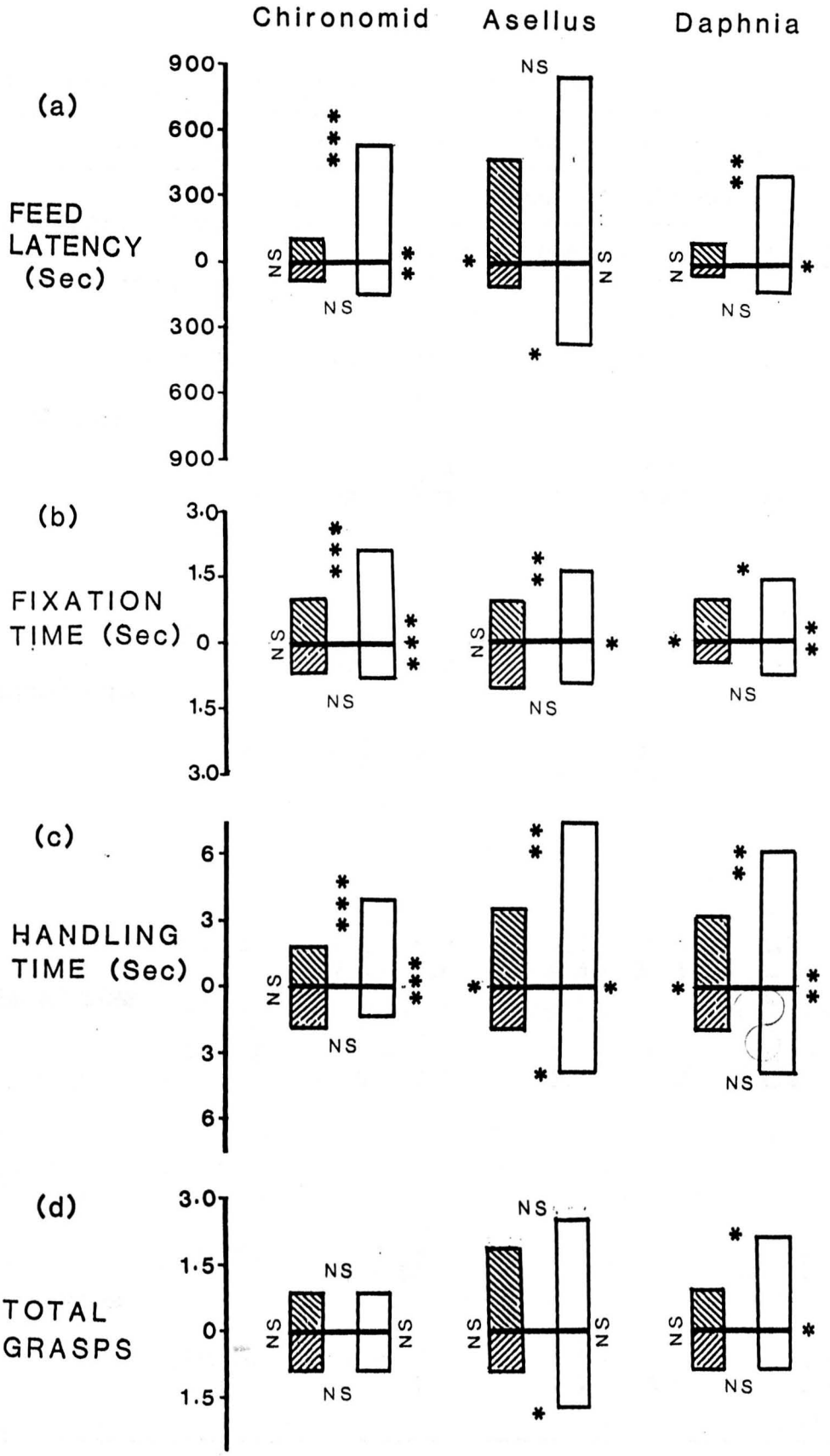

Fig. 3.5: Median scores (the range is given in Table 3.9) of various feeding acts made by wild-caught (lined columns) and laboratory-reared fish (blank columns) during the first test (upper histogram) and the second test (lower histogram).
(* = P <0.05, ** = P <0.01, *** = P <0.001, NS = not significant, U-test)

Table 3.9: Ranges (sec) of scores of various feeding acts made by wild-caught (upper figure) and laboratory-reared fish (lower figure) during the first and the second tests (complementary to Fig. 3.5).

FEEDING ACTS	TEST	RANGE OF SCORES		
		Chironomid	Asellus	Daphnia
FEED LATENCY	First	52 - 273 88 - 1283	40 - 1460 70 - 2523	13 - 591 64 - 1356
	Second	27 - 584 44 - 875	36 - 374 41 - 1056	14 - 188 43 - 791
FIXATION TIME	First	0.4 - 1.5 0.6 - 8.2	0.8 - 3.9 0.7 - 2.3	0.5 - 1.3 0.9 - 2.9
	Second	0.5 - 2.1 0.7 - 1.6	0.4 - 3.7 0.9 - 1.3	0.4 - 1.6 0.4 - 2.4
HANDLING TIME	First	1.4 - 6.3 2.5 - 8.4	2.3 - 5.5 4.4 - 35.1	1.2 - 6.3 1.3 - 26.1
	Second	1.1 - 4.1 1.0 - 2.7	1.1 - 6.2 2.1 - 9.2	0.9 - 4.8 1.2 - 8.0
TOTAL GRASPS	First	1 - 2 1 - 10	1 - 4 2 - 12	1 - 2 1 - 10
	Second	1 - 2 1 - 1	1 - 2 1 - 8	1 - 2 1 - 3

3.3.4 Discussion

The results show clear differences in feeding behaviour between wild-caught sticklebacks, with a full range of experience with the natural diet, and naive, laboratory sticklebacks reared on a highly restricted diet. This is reflected in longer feed latencies (not significant in the case of Asellus), longer handling & fixation times, but (except in the case of Daphnia) not in the number of grasps before eating the prey. The longer latency when feeding on Asellus by both laboratory-reared and wild-caught fish may be due to the fact that Asellus possess the visual cues (eg. movement, colour and shape) which are least preferred by sticklebacks (Chapter 4) and this may delay the feeding responses of the fish. Moreover, Asellus have the lowest energetic contents compared to the other prey used (Table 3.7) and even larger sticklebacks hesitate longer (i.e. spent longer fixation time) before taking Asellus than before taking other prey (Table 3.5). The number of grasps made by the laboratory-reared fish is higher than that made by the wild-caught fish in the case of Daphnia but not in the case of either Chironomid larvae, which have been swallowed from the first grasp, or Asellus which have been spat out frequently by both fish groups. Because Asellus individuals are smaller in size than either Chironomid larvae or Daphnia used, the high spitting-out frequencies to Asellus individuals are unlikely to reflect the difficulty in swallowing them. This indicates that Asellus are indeed undesired prey to sticklebacks.

The longer time spent by naive fish to achieve various feeding acts makes them less efficient foragers than experienced fish. However, the feeding efficiency of laboratory-reared fish has been improved after just one week of experience with the experimental prey, with

the result that most of the differences between the two groups have disappeared. Such improvement has appeared in a reduction in feed latency (not significant in case of Asellus), fixation and handling times, and number of grasps to the prey (Daphnia). These results indicate that, in the case of sticklebacks, recent experience with new food types must be taken into account when assessing the profitability of different diets.

3.4 FEEDING EFFICIENCY OF STICKLEBACK POPULATIONS IN SIMULATED FIELD CONDITIONS

3.4.1 Material and Methods

The fish

The fish used in this test were caught in Loch Lomond and Balmaha Pond and kept in the laboratory for 3 weeks prior to the test. During this time they were fed daily on Tubifex worms. The two groups of fish had standard lengths of 37-40 mm and 36-39 mm respectively.

The tank

Tests were run in two glass tanks (60x25x25 cm), each divided into a resting compartment (12x25x25 cm) and a feeding compartment (48x25x25 cm) by an opaque partition with a sliding door operated from

outside. The first tank was designed to simulate an open water habitat and contained 0.5 cm-thick layer of sand. The other tank was designated to simulate a vegetated habitat and contained a 5 cm-thick substrate with thick bed of vegetation (dominated by Lobellia dortmanna, up to 5 cm long) laid on the bottom. This layer was freshly cut from the bed of Loch Lomond and the invertebrates present were picked up by hand and any possible remaining invertebrates were removed using 'cleaning fish'. The tank was filled with water (20 cm deep) and surrounded with white paper from all sides except a slit for observations.

The prey

The prey used in this test included various species of zooplankton and benthos sampled from the vegetated substrate during July of 1985 (see Table 5.3; Page 122) and with the same densities and sizes (for benthos). Zooplankton were not sized and their size distribution in the tank was assumed to resemble that available in the habitat. The overall prey density in the tank was 950 individuals of zooplankton and 370 individuals of benthos corresponding to 8.4 and 185 mg dry weight respectively. Individual food species were collected from Loch Lomond 24 h prior to the test using either a standard zooplankton-net (for zooplankton) or a hand net (for benthos).

Test protocol

Experimental prey were distributed all over the water surface of the feeding compartment before the start of the test with 2 h in the case of zooplankton and 24 h in the case of benthos. These periods were designated to allow the invertebrates to redistribute themselves in their respective habitats. Following this, each fish (deprived of

food for 24 h) was transferred singly into the resting compartment, allowed to settle down for 30 min, then the door was opened giving it access to the food. The behaviour of the experimental fish was then observed. Recording the data started when the fish crossed into the feeding compartment and began feeding; the time (to the nearest 0.1 sec) spent in searching, fixating, or handling the prey (collectively called feeding time) and the time spent just swimming around or freezing in the tank were recorded. The pattern of movement shown by sticklebacks actively searching for food is quite distinct from that shown during normal swimming; in the latter case the movement of the fish consists of slow-moving bouts using pectoral fins interspersed with relatively long motionless periods. The test was terminated 600 sec after the first prey item had been eaten and the fish was caught, killed, and the prey in their stomachs were identified. To minimize the effect of prey depletion in the tank, the prey that were eaten by each fish were replaced by an equal number of the same species and of similar sizes (in case of benthos) and the test was run on the next fish. Overall, 20 fish from each population were tested, 10 in the zooplankton test and 10 in the benthos test.

Measuring the energetic contents of the food eaten

The energetic content of each prey type eaten by the fish was calculated in the same method described in section 3.2 (Page 35) but using different micro-bomb calorimeter (model AH12/EF), as the other micro-bomb calorimeter, model AH9 (see above), was not available. The number of joules lost in the faeces was estimated and subtracted from the total energetic contents of the prey.

The total number of joules lost in the faeces was estimated in this way: 4 sticklebacks (deprived of food for 48 h) were placed in a tank containing filtered water and a known number of prey of a given type (estimated to be sufficient to satiate the 4 fish) was offered to the fish. Water temperature varied between 13 and 17°C. Thirty six hours after all the prey items had been eaten (this period was thought to be adequate for the faecal material to be egested), the faeces were collected and the feeding procedure was repeated until a sufficient sample of faeces of each prey type was collected. The energetic contents of the faeces were then determined by the same method used for determining the energetic contents of the prey (Page 35).

Calculating the profitability of feeding

The profitability of feeding was calculated for each fish by dividing the number of joules obtained during the 600 sec of the test (excluding the number of joules lost in the faeces) by the number of seconds spent feeding (i.e. 600 sec minus total number of seconds spent in normal swimming or freezing).

3.4.2 Data analysis

A Mann-Whitney U-test was used to test the differences between Loch Lomond and Balmaha Pond fish in the profitability of feeding on zooplankton and that of feeding on benthos, as well as the differences between the profitability of feeding on zooplankton and that of feeding on benthos for each of these two fish groups.

3.4.3 Results

Behaviour of the fish in the feeding compartment

Loch Lomond fish tended to search in the water column, well away from the bottom of the tank, whereas Balmaha Pond fish did the reverse. Even when feeding on zooplankton (water column dwellers), Balmaha Pond fish tended to search in an area of c. 5 cm of the bottom and consumed zooplankton that occurred within this range; only 2 out of 10 fish tested searched and fed in the water column.

Loch Lomond fish usually were able to capture the evasive copepods (the dominant zooplankton in zooplankton-test trial) at the first attempt or otherwise chased such individuals in order to catch them. In contrast, Balmaha Pond fish often failed to capture copepods on the first attempt and in such a case they usually gave up chasing escaped individuals.

Profitability of feeding

Median profitabilities obtained by Loch Lomond and Balmaha Pond fish from feeding on zooplankton and on benthos are presented in Fig. 3.6. Both Loch Lomond and Balmaha Pond fish achieved higher profitability when feeding on zooplankton than when feeding on benthos (this difference was significant only in the case of Loch Lomond fish). Feeding on zooplankton was significantly more profitable for Loch Lomond fish than for Balmaha Pond fish, whereas the reverse was the case when feeding on benthos (but the difference was not significant).

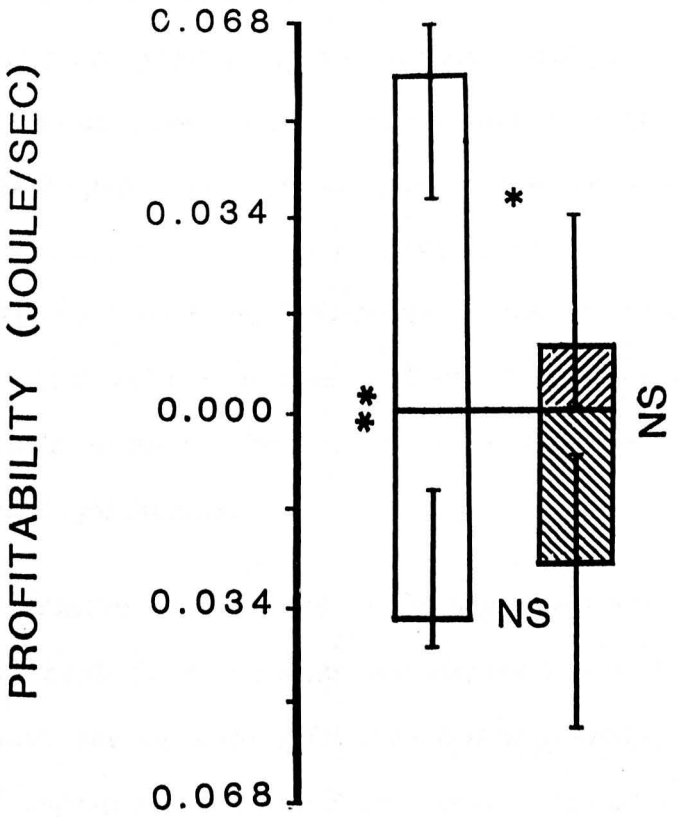

Fig. 3.6: Median profitability (and range) achieved by Loch Lomond (upper histogram) and Balmaha Pond fish (lower histogram) when feeding on zooplankton (blank columns) and benthos (lined columns). (* = P <0.05, ** = P <0.01, NS = not significant, U-test)

3.4.4 Discussion

The results of these experimental comparisons indicate that Loch Lomond and Balmaha Pond fish differ in their efficiency of feeding on zooplankton and benthos. Although the amount of food presented to the fish is much greater in the case of benthos compared to the case of zooplankton (see above), both Loch Lomond and Balmaha Pond fish achieve higher energy intake rates when feeding on zooplankton than when feeding on benthos (the difference is not significant in the case of Balmaha Pond fish). Moreover, when feeding on zooplankton, Loch Lomond fish achieve higher profitability values than Balmaha Pond fish but the reverse is the case when feeding on benthos (this difference is not significant).

The evasive movement of copepods (zooplankton) does not deter Loch Lomond fish from chasing and capturing such prey. However, such movement may be more effective and may enable individual copepods to avoid capture under more complex, natural habitats or under less favourable optical conditions where even a short evasive movement may take the prey out of the visual field of the fish. Thus, the profitability of feeding obtained in this study may be overestimated compared to the true profitability in the field.

The higher achievement of the fish when feeding on zooplankton is likely to reflect the greater accessibility of such prey due to the simplicity of their water-body habitat compared to vegetated habitat (habitat of benthos). In addition, the visual characteristics of zooplankton prey (such as movement and colour) may stimulate sticklebacks to consume such prey readily. These two points are addressed in laboratory and field experiments described in chapters 4 and 5.

The difference between Loch Lomond and Balmaha Pond sticklebacks in their efficiency of exploiting both zooplankton and benthos may be due to the morphological and/or behavioural differences between these two fish groups. Morphology of the feeding apparatus (eg. gillrakers characteristics, mouth size) could make a great contribution to the feeding efficiency of sticklebacks (Bentzen and McPhail 1984, Lavin and McPhail 1986) and if stickleback populations differ in this respect, they are expected to behave differently. Moreover, fish from these two sites may have experienced different food types (zooplankton and benthos) and consequently the foraging success on their respective food types may increase. These possible differences between Loch Lomond and Balmaha Pond fish are investigated in chapter 6.

3.5 CONCLUSIONS

1. Handling time increases from the first to the second prey eaten by sticklebacks. Both fixation and handling times increase with prey size but decrease with fish size. Profitability value increases with fish size and with prey size in the case of Tubifex and Daphnia but not in the case of Chironomid larvae and Asellus.

2. Sticklebacks with full range of experience with natural prey feed faster, fixate and handle the prey within shorter time and require fewer grasps to eat the prey than naive, laboratory-reared sticklebacks. This feeding efficiency can be acquired in the laboratory within a short time (6 days) of experience with the prey.

3. Loch Lomond fish are more efficient at feeding on zooplankton than Balmaha Pond fish which are more efficient at feeding on benthos (this difference is not significant). However, both fish groups are more efficient at feeding on zooplankton than at feeding on benthos (this is significant only in the case of Loch Lomond fish).

CHAPTER 4

DIET CHOICE IN STICKLEBACKS: THE ROLE OF PROFITABILITY AND PROXIMATE VISUAL CUES

4.1 INTRODUCTION

4.1.1 Diet choice in fish

As discussed in the introductory chapter and in chapter 3, optimal Foraging Theory (see Townsend and Hughes 1981, Krebs and Davis 1987 for reveiws) relates foraging patterns in animals to the benefit acquired, usually net energy gain per unit feeding time. In this framework, when given a choice between different prey items, animals are expected to select those items which give the highest rate of energy return (the optimal diet). Many empirical studies have shown this to be the case for fish (Werner and Hall 1974, Ringler 1979, Gibson 1980, Eggers 1982). Werner and Hall (1974) observed that bluegill sunfish feeding on a high density of Daphnia selected the largest and most profitable prey. A similar result was obtained from three-spined sticklebacks when offered a choice between a large and a small Daphnia; the fish took the apparently-larger Daphnia and by doing so achieved higher rate of energetic intake (Gibson 1980).

Fish of various sizes often differ in respect to their food choice. This variation arises mainly because of the difference in mouth size and consequently the difference in the ability of fish to handle larger prey (Unger and Lewis 1983). 0^+ yellow perch (Perca flavescens), changed their diet from Diaptomus minutus to the larger Daphnia pulex when they reached 30-35 mm total length. This shift in diet coincided with an increased ability of young yellow perch to catch the Daphnia (Mills et al. 1984). Accordingly, predators of different sizes may have different optimal prey sizes (Werner 1974, Kislalioglu and Gibson 1975).

Many studies have shown that foragers may associate the profitability value of the prey with some easily distinguished visual characteristic(s) of the prey. In this case, selecting the most profitable prey is based on simple proximate 'rules of thumb' rather than on the actual profitability value of the prey. It has been proposed that the complexity of the habitat and hence the difficulty of direct assessment of the actual profitability favours the use of 'rules of thumb'. Sticklebacks and sunfish assess the profitability values by means of the prey body size (Werner and Hall 1974, Gibson 1980). However, choice on the basis of body size may not always lead to the optimal diet because larger prey might not always be the more profitable one (Bence and Murdoch 1986). Thus, diet selection on the basis of visual characteristics of the prey may or may not lead foragers to the most profitable prey items.

4.1.2 Visual features and prey selection in fish

Vinyard and O'Brien (1976) suggested that prey selection by predators is a result of both predator preference and features of the prey accessibility such as microhabitat selection and predator avoidance. Prey accessibility may well be important when testing predator-prey interactions in the natural habitats, but its importance can be minimized when conducting tests under simple laboratory conditions. Predator preference for a particular prey can be affected by the prey visibility (Curio 1976): movement, colour, size and shape of the prey all contribute to prey visibility to foragers and hence they are expected to be important in prey selection.

Movement

Prey movement has been shown to increase its attractiveness to predators because of the increased conspicuousness of moving prey (Wright and O'Brien 1982). Fifteen-spined sticklebacks (S. spinachia) direct more attacks to moving prey than to stationary prey (Kislalioglu and Gibson 1976 b), and rainbow trout (Salmo gairdneri) are able to locate moving prey more successfully than stationary prey (Ware 1973). Similarly, three-spined sticklebacks prefer a wavy thread to a straight one, but when the straight thread was moved the response of the fish to it increased to a maximum (Meesters 1940).

Speed of movement is also important; both fifteen-spined sticklebacks (Kislalioglu and Gibson 1976 b) and three-spined sticklebacks (Meesters 1940) respond maximally to a prey speed of 3 cm/sec, above which the response declines.

Colour and contrast

Tests carried out on colour preference showed that fish can recognize various colours of the prey, usually the colour of their natural food or those colours that do not match the background. Because natural prey are often cryptic with respect to the colour of their natural habitats, contrast against the background may greatly influence prey detection and consumption by fish. Thus, fish are much more efficient at locating high contrasted prey than at locating low contrasted prey (Ware 1973). Three-spined sticklebacks prefer Daphnia whose colour, pale or red, does not match the background (Ohguchi 1981). Similarly, in a field test, three-spined sticklebacks select the prey (corixids) whose colour makes them conspicuous against the substrate (Popham 1966).

Size

Prey size also affects food choice, with many studies showing that fish select the largest prey available to them (Werner and Hall 1974, Eggers 1982, Main 1985). Prey size also affects the distance at which a prey item can be detected by predators (Eggers 1977). Thus, larger prey can usually be detected at greater distance and consequently elicits more responses.

Shape

Shape of the prey has frequently been reported to influence food choice. Toads (Bufo bufo) respond maximally to a moving strip-like object with long axis in the direction of the movement compared to a squared object with increased edge length (Ewert 1980, cited in Huntingford 1984). Hungry fish often ignore unusually shaped prey (Protasov 1970). In contrast, studies carried out by Main (1985) showed that neither pinfish (Lagodon rhomboides) nor pipefish (Syngnathus floridae) based their diet choice on prey shape.

4.1.3 Relative importance of different visual features in prey selection

Most tests on food preference have been carried out using a simple array of prey. Eggers (1982) and Gibson (1980), for example, used just one species of Daphnia which differs presumably only in body size. However, the case is different in fish foraging in the wild where they face wider range of prey types and consequently many

physical stimuli may be simultaneously presented to them. Prey preference, therefore, may depend on the relative importance of the prey stimuli in eliciting predator response. So, studying the relative importance of the stimuli presented to the fish by the prey helps in identifying those stimuli which produce the highest predator response. This kind of study has rarely been carried out, but Kislalioglu and Gibson (1976 b) provide an example. They studied the relative importance of movement, size, colour, and shape presented by mysid prey to fifteen-spined sticklebacks and found that these stimuli differ in their strength in eliciting the fish response. The relationship of these stimuli was such that movement is the main determinant of diet choice, followed closely by size, then by colour and shape (movement $\geq$ size > colour > shape).

4.1.4 Aims

Therefore, Optimal Foraging Theory predicts that foragers will select the most profitable prey items, but the complexity of the foraging decisions facing predators in their natural habitats may lead them to use simple proximate 'rules of thumb'. Extensive literature on prey selection in fish indicated that various visual cues are involved. Therefore, experiments have been conducted in the laboratory (to avoid the complexity of prey microhabitat choice and predator avoidance by the prey) to investigate the following points:

1. To see if sticklebacks (of various sizes) choose the more profitable of two prey items of different species and/or sizes.

2. To determine, in detail, the proximate visual cues that sticklebacks use to decide what to eat.

3. To investigate the relationship between food selection according to prey profitability (aim 1) and prey visual cues (aim 2).

4.2 THE ROLE OF PROFITABILITY IN DETERMINING PREY CHOICE

4.2.1 Material and Methods

The fish

The fish used in this test were caught in the River Kelvin during January and February 1985 and selected to represent two size groups; small and medium fish with standard lengths of 34-36 mm and 40-42 mm respectively. Thus, they had similar size distributions to the small and medium sized fish used in determining prey profitabilities (Chapter 3). Larger fish were not available. The fish were housed in the laboratory for about two weeks prior to the test and fed daily on chopped earth worms.

The prey

The prey used in this test were of the same species and sizes used for profitability determinations (Table 3.1), i.e. various sizes of Chironomid larvae, Tubifex, Asellus, and Daphnia. The four prey

types differ from each other in their speed of movement, pattern of movement, colour, shape and size and these critical features of the four prey types are shown in Table 4.1. Daphnia have a pale colour, a fast-jerky movement, and a globular shape. Chironomid larvae and Tubifex worms are similar in their appearance and have a red colour, a moderate speed of movement with jerky or smooth pattern, and a straight shape. Even though they are similar in appearance, both Chironomid larvae and Tubifex were used in this test because they provide wider range of profitability values. Asellus have a dark colour, a slow-smooth movement, and a rectangular shape. The sizes of the four prey types are variable.

The test tank

Tests were carried out in tanks similar to those used to test laboratory-reared and wild-caught fish for their feeding efficiency (Page 53) with this modification: two transparent test tubes (with internal diameter of 1.5 cm and external diameter of 1.75 cm) were attatched on the back wall of the choice compartment facing the door. They were at 0.5 cm from the bottom of the tank and at distances of 0.9 cm from each other and 7 cm from the door. The distance between the tubes and the start compartment was short enough for a fish in the start compartment to see the smallest prey used in this experiment. This distance (7 cm) was assessed in a pilot experiment and it agreed with observation by Ohguchi (1981) in that it is short enough for sticklebacks to see the prey. The water level was 7 cm in the tanks and 3.5 cm in the tubes. In addition to the neon tubes providing background illumination in the laboratory, one 60 W bulb was suspended 65 cm above each tank so a diffused and uniform illumination in the whole tanks was achieved.

Table 4.1: The major physical Characteristics of the prey used in determining the role of prey profitability in food selection.

THE PREY	THE FEATURES				
	Speed of movement	Type of movement	Colour	Shape	Size (mm)
Daphnia	Fast	Jerky	Pale	Globular	3.0
Chironomid and **Tubifex**	Moderate	Jerky/ smooth	Red	Straight	6.7 - 28
Asellus	Slow	Smooth	Dark	Irregular/ roughly-rectangular	3.9

The test protocol

24 h prior to each test the fish were transferred from their holding tank into the test tank and fed one meal in the feeding compartment; 6 h before the test they were given another meal to acclimatize them to the test tanks and to train them to expect food in the test chamber. Previous stomach analyses had shown that after 6 h the contents of the stomachs of sticklebacks were markedly digested. Thus, the feeding motivation of the subjects, which is known to influence foraging decisions in sticklebacks (Milinski 1977), was standardized at an intermediate level. Just one hour before the test the fish were removed again into temporary holding tanks.

At the start of each test, two prey items were introduced into the tubes (one in each tube). A stickleback was gently transferred into the start compartment and left there for 5-10 minutes to settle down

before opening the door to give access to the choice compartment. A record was then taken of the tube which was bitten first. The first bite was always followed by a series of bites to the same tube and many fish never paid any attention to the other tube. So the prey contained in the first tube to be bitten was regarded as the preferred prey. Fish which did not react to the prey within 10 min of entering the test chamber were disregarded. The prey and the water in the tubes were changed regularly in order to keep the prey in good condition.

Each size-class of stickleback was tested on six different sets of prey of different types and/or size. The two prey in each set were chosen on the basis of their relative profitability and their appearance. By the end of the whole series of tests, the fish had been presented with prey of a range of profitabilities and with various visual properties. Each fish was tested only once and the tubes were switched regularly in order to minimize any effect of side bias.

4.2.2 Data analysis

A X^2-test was used to test the differences in the first choices of fish to the prey in each pairwise comparison.

4.2.3 Results

Small fish

The preference shown by small sticklebacks for various prey is presented in Table 4.2. Small sticklebacks preferred large Tubifex and medium Chironomid larvae to large Daphnia and small Chironomid larvae respectively (Table 4.2 a); in this case, their preference led them to the more profitable prey items. In contrast, when presented with small Asellus or large Chironomid larvae paired with medium Chironomid larvae (Table 4.2 b), sticklebacks chose the former (though this is not significant) even though they are less profitable. When presented with two prey of similar profitability (Table 4.2 c), the fish regularly preferred one prey type to the other; thus medium Tubifex and large Chironomid larvae were preferred to small Asellus and small Tubifex respectively.

Medium fish

The preference of medium sticklebacks for various prey is presented in Table 4.3. Medium Tubifex and medium Chironomid larvae were preferred to large Daphnia and small Chironomid larvae respectively, while large Tubifex and small Asellus were preferred to small Chironomid larvae (but not significantly so). These choices led medium-sized sticklebacks to the more profitable prey items (Table 4.3 a). In contrast, medium sized sticklebacks preferred large Chironomid larvae to medium Chironomid larvae (this was not significant), even though the latter ones are less profitable. In addition, the fish preferred large Chironomid larvae to small Asellus, even though these prey types have similar profitability values.

Table 4.2: Number of small-sized sticklebacks biting first at various pairs of freely presented prey. (Highlighted prey were the preferred).
*= P< 0.05; ** = P < 0.01; NS = not significant, X^2-test

a. Cases where the more profitable prey was chosen:

Large Tubifex	V	Large Daphnia	
11		3	*
Medium Chironomid	V	Small Chironomid	
9		2	*

b. Cases where the less profitable prey was chosen:

Medium Chironomid	V	**Small Asellus**	
7		14	NS
Medium Chironomid	V	**Large Chironomid**	
6		10	NS

c. Cases where one of two prey of similar profitabilities was chosen:

Medium Tubifex	V	Small Asellus	
14		5	*
Large Chironomid	V	Small Tubifex	
8		0	**

Table 4.3: Number of medium-sized sticklebacks biting first at various pairs of freely presented prey. (Highlighted prey were the preferred).

* = $P < 0.05$; NS = not significant, X^2-test

a. Cases where the more profitable prey was chosen:

Medium Tubifex		V	Large Daphnia	
11			2	*
Medium Chironomid		V	Small Chironomid	
9			1	*
Large Tubifex		V	Small Chironomid	
10			7	NS
Small Asellus		V	Small Chironomid	
16			11	NS

b. Cases where the less profitable prey was chosen:

Medium Chironomid		V	**Large Chironomid**	
6			8	NS

c. Cases where one of two prey of similar profitabilities was chosen:

Large Chironomid		V	Small Asellus	
11			2	*

4.2.4 Discussion

The results obtained from this experiment show that while sticklebacks sometimes choose the more profitable prey item in other cases they choose the less profitable prey item. (Though not significant).

In general, medium-sized sticklebacks show a stronger tendency to select the more profitable prey items than small-sized sticklebacks. Small-sized sticklebacks choose the less profitable prey more frequently, even though such prey do not have the preferred visual features (see below). It may be that the higher nutrient requirements of medium-sized fish impose greater pressure for optimal diet choice. Alternatively, assuming that medium sized sticklebacks are older than smaller ones, experience with food may promote choice of the profitable prey. This possibility was addressed in the experiments described in chapter 3, using sticklebacks from two sites (Loch Lomond and Balmaha Pond) with different invertebrate fauna. Moreover, the larger size of medium fish, compared to small fish, may be a result of their better food selection (i.e. because they are better foragers they grow bigger).

The choices in Tables 4.2 and 4.3 suggest that selection of prey may depend on some physical features of the prey items rather than on the assessment of prey profitability by the fish. In other words, sticklebacks seem to use simple proximate rules in their food selection, which, at least under these conditions, do not always lead them to the more profitable prey item. Red prey items such as Chironomid larvae or Tubifex, for example, were chosen (Tables 4.2 c and 4.3 c), and when the prey were similar in their movement, shape,

and colour (eg. medium Chironomid larvae v large Chironomid larvae ; Tables 4.2 b and 4.3 b), the fish tended to select the larger prey. Thus, experiments described in the following section were conducted to investigate the role of visual cues in prey choice.

4.3 THE ROLE OF VISUAL CUES IN PREY CHOICE

4.3.1 Material and Methods

The specific aims

A series of experiments was designed to alter systematically the major physical stimuli presented by the four prey types used in the previous experiment in order to investigate the effect on prey selection. The specific aims of this series of experiments were:

1. To determine the preferred speed of movement, pattern of movement, colour, shape, and size of the prey. In this context, the importance of prey thickness and prey length in prey selection was investigated. These tests were named as 'single stimulus-dimension tests'.

2. To determine the relative strength of these different dimensions. These tests were called the 'multiple stimulus-dimension tests'.

The fish

The fish used in these tests were caught in the River Kelvin during January and February 1985. Their standard length (40-42 mm) was similar to the standard length of the medium sized fish used in the previous experiment. Fish of this size were chosen because they showed better food selection than smaller fish (see above). The fish were housed in the laboratory for about two weeks under similar conditions to those used in the previous test. During this time, they were fed daily on alternate meals of Tubifex worms, Daphnia, Asellus, and copepods. This mixture was designed to offer the fish food of various shapes, sizes, colours, and movements and also to prevent them from learning specific prey features. It is unlikely that this food affected the fish preference for red colour (the most preferred stimulus for sticklebacks, see below) since fish fed in the laboratory only on pale food (chopped earth worms), for a week after being caught in the wild, still preferred red food to pale food ($n = 20$; $P < 0.01$; X^2-test).

The test tank

The test tanks were the same as those used in the previous experiment (Page 76), but the two test tubes were of wider internal diameter (2.3 cm) to allow a free rotation of the prey inside (see below).

Prey stimuli

Dead Chironomid larvae (killed by 40% formalin) were used in all tests because such prey are easy to handle and manipulate to the desired stimulus configuration. All the chironomids described below

were stiffened by a clear glue in order to minimize the variability in body shape between presentations. The dimensions investigated are shown in Table 4.1 and applied as follows:

a. Movement: various speeds of movement were obtained by variable speed motors which were arranged to rotate the prey on the desired speed. The prey were rotated at speeds of 0, 0.16, 0.48, 0.81, 1.29, 2.59, 3.56, 7.14, and 11.36 cm/sec. Intermediate speeds could not be produced conveniently by the equipments available. These speeds of movements were measured according to the fastest point of the Chironomid, i.e. the head or tail; fish were found to direct their attacks to the head of the prey (Kislalioglu and Gibson 1976 b, and personal observation). Smooth movement is the usual movement of the motors, but to obtain jerky movement, motor gears with 4 depressions on the edge were used.

b. Colour: because the natural red colour of Chironomid larvae changes after killing, red colour was applied by painting the chironomids using red nail varnish. Pale coloured Chironomid larvae are naturally available and this colour stays unchanged by the killing procedure, so pale Chironomid larvae were used when required. Dark colour was obtained by soaking red Chironomid larvae in formalin; this darkened it to a colour similar to that of Asellus.

c. Size: various sizes of Chironomid larvae are naturally available.

d. Shape: Chironomid larvae are naturally straight but rectangular shape was obtained by folding a straight Chironomid larva once from the middle, while globular shape was fashioned by folding the Chironomid larva twice to resemble the shape of Daphnia. In addition, three models of Chironomid larvae were prepared from

wires of the appropriate thickness. These models represent a normal medium Chironomid larva (long thick), a medium Chironomid larva with its thickness reduced to that of a small Chironomid larva (long-thin), and a Chironomid larva with a length reduced to that of a small Chironomid larva (short-thick). The actual measurements (length and thickness) of these prey were shown in Table 3.1.

Prey presentation

The manipulated prey items were presented to the fish in sets of two at a time. Each prey was attached at the middle by a thin thread on a capillary glass tube (1.5 mm in diameter) which in turn was joined onto a shaft (3 mm in diameter) of a variable-speed motor to apply the desired speed. The two prey were then inserted into the test tubes (1.5 cm above the bottom) in such a way to allow them to be rotated freely.

Stimuli presentation

A. Single stimulus-dimension tests:

A complete set of comparisons was carried out between all the configurations within each of the stimulus-dimensions described above (Table 4.1). As mentioned above, the fish were offered a choice between two manipulated prey items at a time. The characteristics of the two items varied according to the purpose of each test: To test the preferred configuration of a stimulus-dimension, the fish were

offered two items which differed only in one respect. For example, to test for the preferred colour, the two items offered differed only in their colour while the speed of movement, pattern of movement, shape, and size were held constant.

To test whether prey choice is affected by the reduction in prey thickness or length, the fish were tested for the choice between a long thick model Chironomid larva and each of a long-thin and a short-thick model.

B. Multiple stimulus-dimension tests:

Having identified the preferred configuration of each stimulus-dimension, the relative importance of different prey dimensions was established by giving the fish a choice between two prey differing in more than one dimension. Taking two stimulus dimensions at a time, one of the two prey items had the preferred configuration for the first dimension while the other prey item had the preferred configuration for the second dimension. For example, to test the relative strength of shape and size, a prey item of preferred shape, but not preferred size, was presented beside a prey item of non-preferred shape but preferred size; the colour and the movement (speed and type) of the two prey items were held constant and were of the preferred configurations. If the prey items of non-preferred shape but preferred size was chosen rather than that of the preferred shape but non-preferred size, then prey shape was regarded as more important than prey size in determining prey choice.

Six possible sets of comparisons were carried out between speed of movement, colour, shape and size. Because the fish did not

differentiate between smooth and jerky types of movement (see below), type of movement was excluded from the comparison. Thus, for convenience, smooth movement of the prey was applied in all the comparisons. In order to investigate the generality of the results, the test was repeated twice more with some modifications:

a. With the preferred configurations of the stimulus-dimensions replaced by the next preferred ones.
b. With the preferred configurations which were held constant replaced by the non-preferred ones.

Test protocol

The fish were tested in the same way as in the previous experiment (Pages 77-78). The preferred prey was again defined as the prey contained in the tube to which the fish made its first bite.

4.3.2 Data analysis

The difference in the number of fish biting first at various prey types was tested using a X^2-tests.

4.3.3 Results

A. Single stimulus-dimension tests

Movement:

There was no significant difference in the first choice of fish to either of the patterns of movement, smooth and jerky ($X^2 = 0.9$; n = 15; df = 1; $P > 0.05$). Up to a speed of 7.14 cm/sec the fish preferred the faster prey (Fig. 4.1). Further increase in the speed caused the fish to stop biting at the tubes and to show fright responses. Of the eighteen fish offered prey moving at 7.14 cm/sec and 11.36 cm/sec only two fish showed any feeding response and they chose the slow prey. A speed of 7.14 cm/sec was therefore regarded as the preferred prey speed for sticklebacks. The actual preferred speed however may lie somewhere between 3.56 and 7.14 cm/sec (the two most preferred speeds given by the motors).

Colour: (Table 4.4 a)

The fish preferred red, pale and dark prey in decreasing order.

Size: (Table 4.4 b)

Medium-sized prey was preferred over small-sized prey.

Shape: (Table 4.4 c, d)

The fish preferred straight, rectangular and globular prey in decreasing order. When offered a choice between long-thick and long-thin prey, the fish selected the two prey at random. In contrast, when offered a choice between long-thick and short-thick prey, the fish preferred the long-thick prey.

The preference order of all configurations of each stimulus-dimension is summarized in Table 4.6.

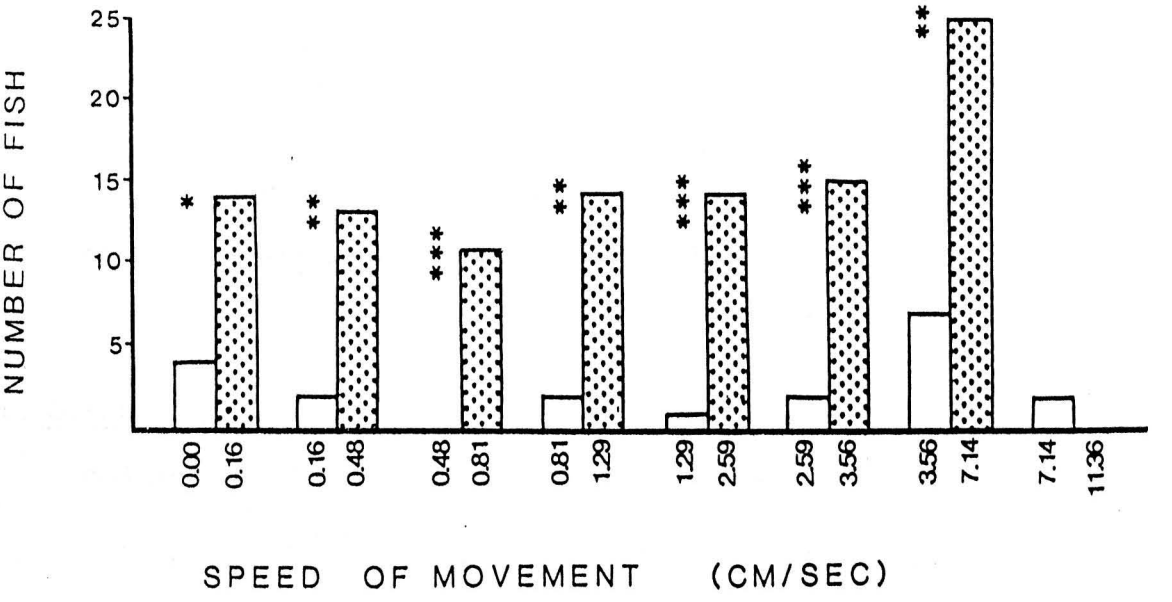

Fig. 4.1: Number of fish biting first at prey moving at various speeds. (* = P <0.05; ** = P <0.01; *** = P <0.001, X^2-test)

Table 4.4: Number of fish biting first at model prey of various types used in the 'single stimulus-dimension tests'. (Highlighted feature was the preferred). ** = P <0.01, *** = P <0.001, NS = not significant, X^2-test)

NO. PREY SELECTED IN EACH COMPARISON		PREFERENCE ORDER
a. COLOUR:		
Red 12	Pale 2 **	Red > Pale
Red 10	Dark 0 **	Red > Dark
Pale 12	Dark 1 **	Pale > Dark
b. SIZE:		
Medium 14	Small 2 ***	Medium > Small
c. SHAPE:		
Straight 15	Rectangular 3 **	Straight > Rectangular
Straight 14	Globular 2 **	Straight > Globular
Rectangular 15	Globular 4 *	Rectangular > Globular
d. THICKNESS and LENGTH:		
Long thick 18	Long thin 12 NS	Thickness is not strong
Long thick 13	Short thick 2 **	Length > Thickness

B. Multiple stimulus-dimension tests

The number of fish biting first at various prey types in the three repetitions of the test is presented in Table 4.5:

a. The case where the preferred configurations of the stimulus-dimensions were used (Table 4.5 a)

The fish preferred the fast prey despite its non-preferred size (small) and disregarded the slow prey despite its preferred size (medium). This indicates that the movement of the prey is a stronger stimulus than its size.

The fast moving prey, regardless of its non-preferred shape (globular), was preferred to the slow moving prey with the preferred shape (straight). So the speed of movement of a prey is a stronger stimulus than shape.

Despite its slow (non-preferred) movement, red prey was preferred to faster moving pale prey (preferred speed). This indicates that red colour of a prey overweighs movement in determining prey selection.

Small-sized red prey, despite its non-preferred size, was chosen rather than medium-sized pale prey (preferred size). This suggests that the colour of a prey is a stronger stimulus than its size.

Red globular prey, despite its non-preferred shape, was chosen rather than pale straight prey which has the preferred shape. This indicates that the colour of a prey overweighs its shape in determining prey selection.

The fish preferred the small straight prey, despite its non-preferred size, over medium globular prey (the preferred size). This suggests that the shape of a prey is more important than its size.

b. The cases where the preferred configurations of the stimulus-dimensions replaced by the next preferred ones (Table 4.5 b) and where the preferred configurations, which were held constant, replaced by the non-preferred ones (Table 4.5 c).

The results of these comparisons are in agreement with those of the previous case (where prey of preferred stimuli were used) with one exception. When red colour stimulus (the preferred colour) was replaced by pale colour stimulus (the next preferred colour) and compared with the speed of movement stimulus, movement became stronger than colour in determining prey choice.

The outcome from these experiments is also summarized in Table 4.6.

Table 4.5: Number of fish biting first at model prey of various types in 'multiple stimulus-dimension tests' when using the preferred configurations (a), the next preferred configurations (b), and when the preferred constant configurations replaced by the non-preferred ones (c): Highlighted stimulus is the preferred. (* = P <0.05; ** = P <0.01; *** = P <0.001; X^2 test)

COMPARISONS	NUMBER OF PREY SELECTED IN EACH COMPARISON					
	(a) (fast=7.14 cm/s; slow=0.16 cm/s)		(b) (fast=3.56 cm/s; slow=0.16 cm/s)		(c) (fast=7.14 cm/s; slow=0.16 cm/s)	
Movement and Size	Slow Medium 0	**Fast Small** 13 ***	Slow Medium 2	**Fast Small** 13 **	Slow Medium 0	**Fast Small** 15 ***
Movement and Shape	Slow Straight 1	**Fast Globular** 14 ***	Slow Rectangular 3	**Fast Globular** 13 *	Slow Straight 0	**Fast Globular** 15 ***
Movement and Colour (1)	**Slow Red** 24	Fast Pale 8 **	Pale Slow 2	**Dark Fast** 13 **	**Slow Red** 15	Fast Pale 0 ***
Colour and Size	**Red Small** 17	Pale Medium 3 **	**Pale Small** 15	Dark Medium 3 **	**Red Small** 14	Pale Medium 1 ***
Colour and Shape	**Red Globular** 20	Pale Straight 4 **	**Pale Globular** 14	Dark Rectangular 4 *	**Red Globular** 15	Pale Straight 0 ***
Shape and Size	**Small Straight** 18	Medium Globular 4 **	**Small Rectangular** 14	Medium Globular 5 *	**Small Straight** 14	Medium Globular 1 ***

(1) The colour is stronger than movement in cases (a) and (c), but the reverse is true in case (b).

Table 4.6: Summary of the preference order shown by three-spined sticklebacks to various stimuli of the prey in the 'Single stimulus-dimension tests' and 'Multiple stimulus-dimension tests'.

STIMULI	PREFERENCE ORDER
a. Single stimulus-dimension tests	
MOVEMENT	Fast (up to 7.14 cm/sec) > Slow
COLOUR	Red > Pale > Dark
SIZE	Medium > Small
SHAPE	Straight > Rectangular > Globular
THICKNESS & LENGTH	Length > Thickness
b. Multiple stimulus-dimension tests	
i. Case where the preferred configurations were used:	Colour > Movement > Shape > Size
ii. Case where the preferred configurations were replaced by the next preferred ones:	Movement > Colour > Shape > Size
iii. Case where the preferred configurations which were held constant replaced by the non-preferred ones:	Colour > Movement > Shape > Size

4.3.4 Discussion

In these experiments, sticklebacks are selecting prey according to the physical appearance. When the prey are similar in appearance but differ in speed of movement, the fish prefer faster moving prey up to a speed of somewhere between 3.56 and 7.14 cm/sec. This preferred speed is higher than the preferred speed obtained by other studies on fifteen-spined sticklebacks, S. spinachia (L.), (Kislalioglu and Gibson 1976 b) and on three-spined stickleback, G. aculeatus, (Meesters 1940) where 3 cm/sec was the preferred prey speed. The fish preference for faster speed in this study (if the difference is of any significance) may be due to the experience of sticklebacks with faster prey in their river habitat, since the true movement of a prey can be expressed by the displacement of the prey by water current (Himsledt and Schaller 1966, cited in Curio 1976). Meesters (1940) concluded that the optimal speed of 3 cm was similar to the speed of movement of the stickleback's prey (Enchytraeus). Similarly, Protasov (1970) reported that non-predatory fish such as damselfish (Chromis chromis), which in the natural environment feed on relatively slow prey, prefer baits with an average velocity of 5 cm/sec, whereas the predatory fish, bass (Serranus scribra), which feed naturally on fast moving fish-prey prefer prey with high velocity (>5 cm/sec).

The fish also prefer red colour to pale colour and both of these colours are preferred to dark colour. Despite the fact that red colour appears more conspicuous than pale colour against the white background of the test tank, the fish preference for red colour is unlikely to reflect such conspicuousness. Dark colour is more conspicuous than pale colour, yet in this case the pale colour is the preferred. This suggests that this preference is for colour per se, rather than for the contrast against the background.

Sticklebacks distinguish between various shapes of prey, with straight shape being preferred to rectangular shape, and both of these being preferred to a globular shape. Given that the prey of different shapes differ both in length and thickness, two explanations (which are complementary and not mutually exclusive) may be given to this sequence of preference. First, since handling time increases rapidly with increasing prey thickness above a threshold (0.98 mm, Chapter 3), and folding the medium Chironomid larvae (0.79 thickness, Table 3.1) increases their thickness to well above this threshold, sticklebacks may have sought to avoid prey of long handling times by choosing the longer and thinner prey. Second, since reduction in prey length, regardless of prey thickness, reduces the attractiveness of prey in these tests (Table 4.4 d), and assuming that this applies to thicker prey, prey length may become the key feature. The shape preference may therefore correlate with the fish preference for prey of different lengths. The latter explanation may also apply to prey size selection in this test, since medium Chironomid larvae, which are longer than small Chironomid larvae, were the preferred.

Sticklebacks prefer larger prey to smaller prey. Preference for larger prey by planktivorous fish has been shown in other experimental studies (Werner and Hall 1974, Gibson 1980, Eggers 1982). The present result expands the conclusion of these studies by showing that large size is preferred in sticklebacks feeding on benthic prey such as Chironomid larvae.

Of the features that determine prey choice, colour and movement of the prey are the most important stimuli, but there is an interaction between these two effects. Colour is a stronger stimulus than movement when the prey are red but the reverse is true when the red

prey are replaced by pale prey. Thus, the sticklebacks show the strongest responses to red colour. This preference for red colour may be inherited or may be due to the experience of the fish with red food (red Chironomid larvae and Tubifex) in their natural habitat, since such food is available in the same site where the sticklebacks were caught (Pers. Observ.). Labass (1959, cited in Protasov 1970) found that colour reaction in young fish develops during the early stages of growth and continues to do so for some months indicating that colour reaction can be influenced by experience. It was attempted in this study to test the effect of experience on sticklebacks preference for certain colours (as well as other visual features) by rearing fish on colour-controlled diet, but unfortunately every attempt failed to get them respond to artificial prey.

The shape of the prey is less important than either colour or movement in determining diet choice. Similarly, prey shape is the least important feature in determining prey selection in fifteen-spined sticklebacks (Kislalioglu and Gibson 1976 b). Again, prey morphology does not influence preference in pinfish or pipefish (Main 1985), which base their selection on prey movement. However, the role of prey shape in determining diet choice in pinfish and pipefish should not be ruled out since, as the present study has shown, prey movement is a stronger stimulus than prey shape and the fish would select the moving prey regardless of its shape.

Prey size is found to be the least important feature in determining diet choice of sticklebacks. This finding does not agree with that obtained for fifteen-spined sticklebacks (Kislalioglu and Gibson 1976 b) where prey size was the strongest stimulus (compared to colour and shape). However, Kislalioglu & Gibson's experiment has been

conducted in a much larger experimental tank (approx. 47X31X31 cm, i.e. more than ten times larger than the tank used in my experiment, see above) and used approximately similar prey sizes (7 mm and 14 mm-long) to those used in this study. Thus, these experimental situations may require the fish to detect the prey from a longer distance and consequently the size of the prey becomes a critical issue in determining the preference.

4.4 PREY PROFITABILITY AND PREY VISUAL CUES RELATIONSHIP

It is shown in the study of the role of profitability in prey choice (section 4.2) that sticklebacks select certain prey types regardless of the profitability. Medium Tubifex, and medium & large Chironomid larvae, for example, are preferred to large Daphnia, small Chironomid larvae, and small Asellus respectively (Table 4.3). When the role of visual cues in prey selection in sticklebacks is investigated systematically (section 4.3), a significant preference for red colour, fast movement, straight shape and larger sizes of the prey is found. The relative strength of these stimuli is that: colour is stronger than movement (when the prey are red, but the reverse is true when the prey are pale) followed by shape and then by size.

These two sets of results can be integrated: the preference for medium Tubifex to large Daphnia by sticklebacks may be due to the fact that medium Tubifex have the preferred colour (red), the preferred shape (straight), and presumably the preferred size (large).

The preference for medium Chironomid larvae over small Chironomid larvae might be due to the large size of the former (colour, movement, and shape are similar). Similarly, large Chironomid larvae were preferred to small Asellus and this preference may be because that large Chironomid larvae have the preferred features, i.e. red colour, faster movement, straight shape, and larger size.

Thus, the results of the profitability choice tests have arisen because sticklebacks have chosen the prey with the preferred stimulus on one or more dimensions. In other words, they seem to use simple proximate rules to choose their food. In some cases these rules lead sticklebacks to the more profitable prey item (Table 4.3 a) while in other cases (Table 4.3 b) they do not do so or even lead them to the less profitable of two prey items. Many other laboratory experiments of extreme simplicity (eg. Gibson 1980) have shown that fish prefer the more profitable prey even though the fish base their selection on simple rules, i.e. prey size.

However, the situation of prey selection in nature may differ from that in the laboratory in many respects. The major differences arise from:

a. Differences in prey accessibility due to microhabitat choice and predator avoidance of the prey.
b. Differences in visibility conditions due to lower light levels which may give different emphasis to different dimensions.
c. Differences in feeding behaviour due to the presence of competitors.

Thus, experiments were carried out in chapter 5 to investigate prey selection in the field, taking into account various aspects that may influence such selection.

4.5 CONCLUSIONS

1. Sticklebacks do not always select their food according to the prey profitability values as defined by Energy/handling time.

2. Sticklebacks do recognize various visual stimuli of the prey and do prefer one to another. They show a preference, in decreasing order, for red, pale, and dark colours, for fast (up to 7.14 cm/sec) and slow movements, for straight, rectangular, and globular shape and for medium and small sizes. However, sticklebacks do not differentiate between smooth and jerky movements of the prey. The relative strengths of the above stimuli are that: colour (when red) is stronger than movement, followed by shape, and then by size. When red colour is replaced by pale colour, a different preference order is found; i.e. movement becomes stronger than colour, followed by shape and then by size.

3. Food preference of sticklebacks according to prey profitability and prey visual cues can be integrated. The fish select the prey which possess one or more preferred stimuli, indicating that sticklebacks use simple proximate rules to select their food. These rules, in some cases, lead the fish to the more profitable prey but in other cases do not do so.

CHAPTER 5

FIELD STUDIES OF PREY AVAILABLILITY, STOMACH CONTENTS AND PREY PREFERENCE OF STICKLEBACKS

5.1 INTRODUCTION

5.1.1 Diet choice of fish

One of the central questions facing foraging predators is which of the many prey items that they encounter should be included in their diet. Two distinctive strands can be identified in the existing vast literature on this subject. The most recent of these strands (Optimal Foraging Theory) involves theoretical analyses of the costs and benefits of observed behaviour of predators leading to testable predictions of food selection to see if the foragers are designed to maximize potential currencies (MacArthur and Pianka 1966, Werner and Hall 1974, Krebs 1978, Werner et al. 1981). In contrast to this functional approach, the other strand, going back much further, includes empirical studies of what is actually selected by foragers in their natural habitats, often employing one of a variety of electivity indices to relate the food eaten to that actually available in the habitat (Cock 1978, Lechowicz 1982). This latter approach is of a great importance in gaining an understanding of the characteristics of predator, prey and environment that act as proximate determinants of the observed pattern of selection.

Selection among prey types by a predator may be the result of a simple preference or differences in prey accessibilty which might result from differences in habitat selection and predator avoidance of the prey (Vinyard and O'Brien 1976). The most important determinants of prey risk are prey exposure (visibility and vulnerability to predators), colour, activity, size, density and palatability (Ware 1973, Hyatt 1979). In addition, the presence of feeding competitors may affect food choice (Milinski 1986 a). These factors are discussed in turn below:

Prey exposure

Prey exposure is often related to the availability of physical refuges and physical barriers available to the prey (Zaret 1980). Zooplankton inhabiting the water body, may be more exposed to predation risk than organisms inhabiting the structured lake-bed (benthic organisms). Increased physical structure of the habitat reduces prey encounter rate and consequently reduces prey risk by increasing searching and handling times (Anderson 1984).

Prey colour and contrast

Prey colour and contrast against the background may also increase prey vulnerability. Three-spined sticklebacks feeding in natural habitats consume prey whose colour makes them conspicuous against the substrate (Popham 1966). Moreover, the degree of pigmentation is found to be important in determining the level of predation on cladoceran by planktivores (Zaret 1972). The black pigmentations associated with the compound eyes or digestive tracts provide great contrast against the transparent body of many cladocerans. Brook sticklebacks (Culaea inconstans) preferentially consume black-pigmented Daphnia rather than the unpigmented copepods (Tompkins and Gee 1982).

Prey activity

Prey activity increases the reaction distance of predators (Wright and O'Brien 1982) and consequently increases the chance that the prey will be recognized and consumed (Zaret 1980). However, the evasion responses of the prey can have the converse effect. Copepods avoid

predation by sunfish (Lepomis macrochirus) because of their high ability to escape the visual field of the fish (Vinyard 1980). Feeding on less evasive prey is in accordance with the prediction of Optimal Foraging Theory, since such prey require shorter handling time (Eggers 1977) and predators therefore can maximize their rate of successful captures.

Prey size

The importance of body size in prey selection by planktivores has been investigated for zooplankton (Brooks 1968, Gibson 1980) and benthos (Ware 1973, Main 1985) and the results have shown that prey risk is directly proportional to body size. However, it is difficult to separate the contribution of body size to prey selection from other associated features. For example, within the Cladocera, large individuals have conspicuous eye pigmentations (Zaret 1980) which also play an important role in prey selection. Many workers have therefore concluded that body size per se does not adequately explain prey selection by fish (Zaret and Kerfoot 1975). Blueback herring (Alosa aestivalus) feed predominantly on the small Bosmina longirostris rather than on the large Diaphanosoma brachyurum which have transparent bodies with greatly reduced compound eye pigmentations (Burbidge 1974).

Prey density

Increased prey density may lead predators to concentrate their feeding efforts on the most abundant prey (Werner and Hall 1974). This situation may reduce searching time and may lead to forming 'search image' (Lawrence and Allen 1983). That is, foragers 'learn to see' prey items which are similar to one consumed earlier. Predators

may also pay a cost when feeding on high density prey items of similar nature due to the confusion effect (Bertram 1978), but the overall feeding rate may still be higher than when feeding on solitary individuals (Heller and Milinski 1979). Selecting high density prey has been shown to increase the feeding efficiency of foragers (Ware 1971, Werner and Hall 1974).

Prey palatability

Palatability of the prey may play an important role in prey selection (Hyatt 1979). Some prey species (e.g. water mites) are distasteful to predators due to the noxious chemicals they produce and are therefore avoided by fish (Zaret 1980). Small water mites are still consumed by many fish (Pieczynki and Prejs 1970) which could be due to the difficulty of recognizing such organisms or to insufficient production of noxious chemicals (Hyatt 1979).

Competitors

The presence of competitors can alter food selection. The presence of roach (Rutilus rutilus) strongly affects the diet of the coexisting perch (Perca fluviatilis) in several South Swedish Lakes, resulting in a strong decline in the abundance of the latter fish (Lessmark 1983). Roach exploit small zooplankton more efficiently than perch. When deprived of zooplankton in this way, perch are forced to shift their diet to include benthos.

Thus, various features of the prey as well as the presence of competitors are involved in determining prey selection. However, none of these features is the decisive factor; the total contribution of all features of the prey determines its vulnerability to a predator (Zaret 1980).

5.1.2 Diet of three-spined sticklebacks

As mentioned in the introductory chapter, previous studies on the diet of three-spined sticklebacks have revealed that this species eats a wide variety of zooplankton and benthos. However, the diet of sticklebacks may vary greatly between habitats, and from time to time in the same habitat, depending on the type of food available to them (Wootton 1984). Previous work on the seasonal variation in the diet of sticklebacks has concerned mainly with the analysis of stomach contents (eg. Hynes 1950, Allen and Wootton 1984, Ukegbu 1986) without referring to the type of food available in the environment. A notable exception to this generalization is provided by Moore and Moore (1976 a) who filtered sticklebacks from the cooling intake of the Oldbury-on-Severn nuclear power station and sampled the food available in the main water (Moore and Moore 1976 b) to study food selection, but unfortunately all the sticklebacks sampled had empty stomachs. Manzer (1976) used the published data of LeBrasseur and Kennedy (1972) on the food availability in his study area (Great Central Lake, Vancouver) to compare it with the data of the food eaten by sticklebacks in order to study food selection. Such studies did not monitor precisely the food available to the fish whose stomachs were analysed. Again, these studies did not relate food choice to the microhabitats to understand the foraging decisions of fish as they occur in the wild.

Regarding that the food available to sticklebacks is variable in space and in time, some potential foraging patches are likely to be more favourable than others, and a patch that is good on one occasion may be poor on others. Sticklebacks in their natural habitats are therefore faced with foraging decisions whose complexity depends on how variable their food supply is. Fine scale sampling is therefore

needed to detect the kind of foraging decisions which sticklebacks need to make when foraging in their natural habitats.

5.1.3 Competition with ten-spined sticklebacks

Sticklebacks are known to compete for food with many coexisting fish species such as juvenile sockeye salmon, Oncorhynchus nerka (Manzer 1976) and ten-spined sticklebacks, Pungitius pungitius (Wootton 1984). Comparative studies on the diet of three-spined sticklebacks and the commonly coexisting species, ten-spined sticklebacks, have shown conflicting results. In River Birket (north-west England), for example, the two species have almost identical diets, comprising mainly higher Crustacea and Copepoda (Hynes 1950). In contrast, three-spined sticklebacks in Matemak Lake (Quebec) eat primarily zooplankton whereas ten-spined sticklebacks eat cladocerans and chironomids from the vegetated regions of the lake (Coad and Power 1973; cited in Wootton 1984). The potential competition between three-spined sticklebacks and the coexisting fish species such as ten-spined sticklebacks seems to depend on the locality and/or the availability of food.

5.1.4 Aims

The study described in this chapter is designed to investigate prey selection by three-spined sticklebacks in the natural habitats and to relate this to the prey types available as they vary with time and space. The specific aims are therefore:

1. To survey the food available to sticklebacks in natural habitats of different structural complexity and to study the variability of such food in space and time.

2. To analyse the diet and to investigate prey selection of sticklebacks feeding above different substrates and at different times.

3. To determine the role of various prey features (movement, density, etc.) in diet selection and to interpret the sticklebacks food selection in the light of the existing knowledge of fish behaviour.

4. As a subsidiary aim, to study prey size selection by sticklebacks.

5. To study the potential competition between three-spined sticklebacks and the coexisting ten-spined sticklebacks.

5.2 DESCRIPTION OF THE STUDY SITE

This work was carried out in Camas An Losgainn Bay (NS 373 957, National grid reference) of Loch Lomond. The bay forms an area of 50X100 m of the east shore of the Loch with a gradually-declining smooth bottom. This site was chosen because it is conveniently situated near the Universities Field Station at Rowardennan. It is well known to be a natural habitat for sticklebacks and it offers various types of substrates. Three types of substrates were chosen for this study:

a. Vegetated substrate: consisted of silted sand covered with a sward bed of dark green submergent aquatic plants dominated by Lobelia dortmanna which grew to an even height of c. 5 cm.

b. Stony substrate: composed of stones of various sizes (up to 60 cm^2) imbedded in sand and silt and sparse individuals of L. dortmanna grew over this substrate.

c. Sandy substrate: consisted of bare sand with neither vegetation nor gravel.

The selected patches of these three substrates were adjacent to each other and covered areas of 60, 60, and 40 m^2 respectively. The water depth above the study substrates ranged from 80 to 95 cm. Preliminary work during the summmer of 1984 had indicated that the greatest variety of food taxa and the largest number of individuals occurred at this depth. The water was clear during the whole period of study. Water temperature varied from 12 to 16°C and oxygen saturation ranged from 82 to 100 % (measured by Mackereth combined Thermo-Oxygen meter).

5.3 DISTRIBUTION OF THE FOOD OF STICKLEBACKS

5.3.1 Material and Methods

Sampling schedule

Quantitative samples of zooplankton and benthos (i.e. the invertebrates that live in the water column and lake bed respectively) were collected from the site on three occasions: 23rd June, 15th July,

and 12th August 1985. Both, June and July samples included the three substrates mentioned above (i.e. vegetation, stones, and sand), but the sandy substrate was not sampled during August due to unfavourable weather conditions.

Determination of sample size

A representative sample was worked out after taking a number of sub-samples. The representative sample was defined by stabiliz ing both the number of taxa and their abundance over the successive subsamples (Elliott 1983). The first criterion was assumed to be met when three successive sampling units brought no more new taxa into the total list (Elliott 1983). The second criterion was tested by using X^2-tests on the number of individuals of the common taxa across the sub-samples. A non-significant difference between the sub-samples was taken to indicate stabilization of the abundance of the taxon under consideration. This method of determining sample size was used rather than the traditional way of using the diversity measure because the latter method requires large sample size which is too laborious. These two criteria were applied on zooplankton and benthos living on the three substrates mentioned above. Some benthic taxa (eg. Glassiphoniidae) are known to be rare on sandy substrate and their occurrence is accidental, such taxa were therefore disregarded when determining sample size above sand.

A representative sample of zooplankton was determined by filtering 7 random subsamples (4 l each) collected from the water above each of the three substrates. It was found that 5 subsamples (i.e. 20 l water) were enough to represent the zooplankton above each substrate.

A representative sample of benthos was worked out by taking 7, 6, and 4 random sub-samples from vegetated, stony, and sandy substrates respectively. It was found that 5, 4, and 3 subsamples were enough to represent the benthos on vegetation, stone, and sand respectively.

Sampling procedures, sorting and identification

a. Zooplankton:

Zooplankton samples were collected from various depths and locations of the water above each substrate and filtered through a standard zooplankton mesh. Zooplankton were washed away carefully by spraying water from a squeezing bottle and then preserved in labelled vials. Preserved zooplankton were later transferred into 25 cm^3 counting chamber containing water and left for 2 h to settle. Zooplankton were then identified to the lowest possible taxon and recorded as number per 20 l water.

b. Benthos:

Samples of benthos were taken on the same occasion of collecting samples of zooplankton. They were collected using trays (25X25X5 cm) with transparent perspex bases and metal-mesh walls (16 mesh/cm^2) to facilitate colonization of organisms with burrowing habit such as oligochaets (pilot experiment). The trays were buried in the substrate by digging a hole of appropriate size in the substrate, sinking the tray in the hole and filling it with the excavated material and associated fauna. The

surface of the trays matched that of the surrounding substrate. This method of sampling benthos was chosen because the substrates to be sampled (especially stony substrate) were too hard to allow grab or core samplers to be used.

The trays were left in their places for at least three weeks, by this time they were packed with invertebrates. Following colonization, each tray was covered with a metal lid to protect the sample and then lifted up with the help of attached handles. As the sample was lifted above the substrate, a net (1 m in diameter) made of fine mesh (360 mesh/cm^2) was placed under the tray to trap any animals freed during lifting. The contents of each tray were first washed in the Loch through a sieve (400 μ) and any stones were picked out and the attached animals washed out carefully above the sample.

In the laboratory, the samples were washed again under tap water until the run off water was clear. Green plants were separated by hand because they float and interfere with separating the animals (see below). They were washed above the sample and placed in a white tray with water, then the attached animals were picked up by hand. The residue (usually detritus and sand) carried the vast majority of the invertebrates so that these organisms were separated using floatation technique. Magnesium sulphate (Mg SO4 1.15 s.g., Weerekoon 1953) was added to the residue in a ratio of 8:1 (Mg SO4 : residue). The sand particles sank directly and once the detritus absorbed water they also sank leaving the invertebrates on the surface of the solution. The invertebrates were picked up individually using a bent needle. The residue was stirred frequently to allow the clinging or accidentally imprisoned animals to be freed on the surface. Stirring the residue continued until three consecutive stirrings brought no single animal on the surface. Most of the animals were separated at

this stage but it was necessary to check for the cased caddis larvae (which did not leave their case and stayed submerged) and molluscs which do not float. Such animals were checked for and picked out individually by hand. The benthic invertebrates were sorted out when they were alive because that their movement made sorting more efficient.

As the animals were picked up they were separated into their major taxonomic groups (according to their appearance) and then preserved. This saved much time later on while identifying the preserved animals. The abundances of benthic taxa in the aggregated samples were determined. Estimates of the total number of individuals per sample (i.e. per 0.312, 0.250, and 0.187 m^2 of vegetation, stones and sand respectively) and hence per square metre were obtained.

5.3.2 Data analysis

Shannon-Weaver (1949) diversity indices, H, was calculated for each aggregated sample as:

$$H = - \sum P_i \ln P_i$$

where:

P_i = the proportion of food type i in the sample.

The diversity measure represents both number of food types present in the sample and the evenness of their distribution. Thus, a community with many, evenly represented food types will have higher diversity value than a community with few, unevenly represented food types. However, this measure is ambiguous because it depends on two

variables (number of food types and their evenness). It is, therefore, worth determining the evenness, J, of the food types in question. This was obtained using the formula:

$$J = H/ \ln s$$

where:

s = the number of food types in the total sample (Pielou 1975).

Strictly speaking, the above mentioned indices should be applied to situation where 'total number of species' in the sample is accurately determined, the uncommon event in most ecological studies (Pielou 1975). In the present study, such indices were applied on a set of food types (a combination of many levels of taxonomic classification) in order to follow the changes over time and space and as the level of classification of a given food type was constant, any bias introduced should be constant and should not alter the pattern of changes. However, the results obtained here may not be strictly comparable to other workers and to avoid the confusion the indices were called 'prey diversity' and 'prey evenness' instead of 'species diversity' and 'species evenness' respectively.

In order to detect the important trends of food variability and to make statistical comparisons between samples, rare taxa were grouped either together or with the common taxa (Table 5.2). This was usually done according to the taxonomic relativeness and ecology of the prey (similarity in appearance, activity and habitat). Differences in the abundance of a given taxon between samples were tested using X^2-tests.

5.3.3 Results

Prey available

Various taxa of zooplankton and benthos found in the study site are presented in Table 5.1.

a. Zooplankton:

Eleven different taxa of zooplankton were identified from the site during the period of study, with 7-9 taxa present at any one time. The most common prey types were *Bosmina coregoni*, *Diaptomus spp.* and *Cyclops spp.* but their abundance varied according to both substrate type and months.

b. Benthos:

Thirty seven different taxa of benthos were identified during the period of study. Twenty-two to twenty-eight taxa were recorded at any one time on both vegetation and stones, whereas 15-16 taxa were recorded on sand. Oligochaeta and Chironomid larvae were the most common benthic prey types.

Substrate differences

The distribution of zooplankton and benthos according to substrate type is presented in Table 5.3. Community characteristics of zooplankton and benthos are presented in Tables 5.4 and 5.5 respectively.

a. Zooplankton:

Zooplankton densities were similar in the water column above vegetation and stones and were higher than that found above sand, although the number of prey types is similar above the three substrates. This is mainly due to the lower density of B. coregoni (during June) and Diaptomus spp. (during July) above the sandy substrate. However, prey diversity and prey evenness indices were higher in the water above sand than in the water above either vegetation or stones. This reflects the fact that the guilds above vegetation and stones were dominated by one single prey type more distinctively than the case above sand.

b. Benthos:

The overall density of benthos decreased in the sequence vegetation - stones - sand and the abundances of the vast majority of benthic prey types decreased accordingly. Number of prey types, prey diversity and prey evenness were higher on vegetation and stone than on sand.

Monthly differences

The abundance of various groups of prey in each month of the study period is presented in Table 5.6 (rearranged from Table 5.3 to highlight the time effect):

a. Zooplankton:

Zooplankton density decreased as the summer progressed towards August. This is mainly due to the decrease in abundance of B. coregoni (from June to July and August) and Diaptomus spp. (from July to August). The abundance of Daphnia sp. also decreased from June to August whereas Chydoridae rapidly increased towards August. Cyclops spp. had its highest density during June then declined during July and August. Prey diversity and evenness indices increased as the time progressed towards August indicating that zooplankton guilds above the three substrates were dominated by more prey types during August.

b. Benthos:

Generally, the density of benthos increased from June to July and August (except on vegetation, where it decreased from June to July). This is mainly due to the increase in the abundance of Plecoptera, Chironomid larvae and pupae, Oligochaeta, and Eurycercus lamellatus. Caenis sp., Ephemerella ignita and caddis larvae either decreased sharply in number or disappeared in the sample taken during August. Prey diversity and evenness indices on both vegetation and stones decreased from June to August. This is due to the more evenly represented prey types in June than in July and August. On sand, the values of such parameters were similar between June and July.

Table 5.1: The invertebrate taxa sampled in Loch Lomond during the period June-August 1985.

a. ZOOPLANKTON

Cladocera
- Sididae
- *Chydorus* sp.
- *Alona* sp.
- *Bosmina coregoni* (Baird)
- *Daphnia* sp.
- *Leptodra kindti*
- *Polyphemus pediculus*
- *Bythotrephes longimanus*

Copepods
- *Diaptomus* spp.
- *Cyclops* spp.
- Nauplii

b. BENTHOS

ARTHROPODA

Insecta-nymphs

Ephemeroptera
- *Caenis* sp.
- *Ephemerella ignita* (poda)
- *Ephemera danica* (mull.)
- *Cloeon simile* Etn.
- *Centroptilum luteolum*
- *Ecdyonurus dispar*

Plecoptera
- *Leuctra nigra*
- *Leuctra hippopus*
- *Nemora erratica*

Hemiptera
- Corixidae

Insecta-adults
- *Oulimnius* sp.
- *Deronectes depressus*
- Other Coleoptera

Subimagines

Insecta-larvae

Coleoptera
- Elmidae
- *Dryops* sp.
- *Hydroporus* sp.

Diptera
- Tipulidae
- Chironomidae
- Ceratopogonoidae
- Other Diptera

Trichoptera
- *Limnephilus* sp.
- *Sericostoma personatum*
- *Polycentropus flavomaculatus*

Insecta-pupae
- Chironomidae
- Other Dipteran

Crustacea

Isopoda
- *Asellus aquaticus* (L.)

Amphipoda
- *Gammarus pulex* (L.)

Cladocera
- *Eurycercus lamellatus*

Ostracoda

ANNELIDA
- Oligochaeta
- Glassiphoniidae (leech)

PLATYHELMINTHES
- Triclads (flat worm)

Nematoda

Arachnida
- Hydrocarina (water mite)
- Spider

MOLLUSCA
- Sphaeriidae
- Gastropoda

Table 5.2: The constituents of various groups of prey used in the study of the variability of zooplankton and benthos in the study site.

GROUPS	COMPOSITIONS
ZOOPLANKTON:	
Chydoridae	Chydorus sp. + Alona sp.
Bosmina coregoni	B. coregoni
Daphnia sp.	Daphnia sp. + Sididae
Polyphemidae	P. pediculus + L. kindti + B. longimanus
Diaptomus spp.	Diaptomus spp. + Nauplii
Cyclops spp.	Cyclops spp.
BENTHOS:	
Caenis sp.	Caenis sp.
Ephemerella ignita	E. ignita
Ephemera danica	E. danica
Other Ephemeroptera	C. luteolum + C. simile + E. dispar + Corixidae
Plecoptera	L. nigra + L. hippopus + N. erratica
Coleoptera adults	Oulimnius sp. + D. depressus + Other Coleoptera
Coleoptera larvae	Elmidae, Dryopus sp., Hydroporus sp.
Asellus aquaticus	A. aquaticus + G. pulex
Chironomid larvae	Chironomid larvae+ Tipulida + Other Diptera larvae
Chironomid pupae	Chironomid pupae+ Subimagines + Other Diptera pupae
Ceratopogonoid larvae	Ceratopogonoid larvae
Oligochaeta	Oligochaeta
Glassiphoniidae	Glassiphoniidae+ Triclads + Nematoda
Arachnida	Hydrocarina + Spiders + Ostracoda
Mollusca	Sphaeriidae + Gastropoda
Caddis larvae	Limnephilus sp.+ S. personatum + P. flavomaculatus
Eurycercus lamellatus	Eurycercus lamellatus

Table 5.3: *Number of zooplankton and benthos sampled above vegetation, stones and sand during summer 1985. Note: the figures represent number of zooplankton per 20 l water or number of benthos per sample, i.e. per 0.312, 0.25, and 0.187 m^2 for vegetation, stones, and sand respectively.*
(* = P <0.05; ** = P <0.01; *** = P <0.001; NS = not significant; X^2-tests).

PREY TYPES	JUNE				JULY				AUGUST		
	VEGETATION	STONE	SAND		VEGETATION	STONE	SAND		VEGETATION	STONE	
ZOOPLANKTON											
Chydoridae	7	2	5	NS	5	6	12	NS	65	49	NS
Bosmina coregoni	2850	2820	1560	***	28	17	41	**	60	96	**
Daphnia sp.	12	2	5	*	5	1	1		1	1	
Polyphemida	5	1	14	**	4	1	6		2	10	*
Diaptomus spp.	22	35	30	NS	745	624	244	***	34	45	NS
Cyclops spp.	44	61	11	***	6	17	4	**	11	4	NS
BENTHOS											
Caenis sp.	107	30	3	***	15	7	0	**	0	0	
Ephemerella ignita	54	10	2	***	10	17	0	***	0	1	
Ephemera danica	7	2	0		27	0	0	***	2	4	
Other Ephemeroptera	4	5	0		9	2	1		13	5	NS
Plecoptera	31	36	1	***	16	33	6	***	102	57	*
Coleopterian adults	48	22	0	***	16	24	3	**	20	37	**
Coleoptera larvae	169	69	4	***	45	87	3	***	93	63	NS
Asellus aquaticus	23	13	4	*	8	32	1	***	16	11	NS
Chironomid larvae	128	38	15	***	75	187	22	***	743	241	***
Chironomid pupae	27	3	2	***	7	6	1		56	24	**
Ceratopogonoid larvae	126	14	76	***	76	36	51	**	67	12	***
Oligochaeta	357	128	23	***	553	157	63	***	409	205	***
Glassiphoniidae	32	4	1	***	44	9	0	***	28	12	NS
Arachnida	11	4	1	NS	7	4	1		12	7	NS
Mulscus	1	0	7		13	1	6	*	3	2	
Caddis larvae	34	8	7	***	20	7	1	**	1	1	
Eurycercus lamellatus	25	17	0	***	25	16	0	***	44	32	NS

Table 5.4: Characteristics of the zooplankton above vegetated, stony, and sandy substrates from Loch Lomond during June, July, and August 1985.

DATE	SUBSTRATE TYPE		
	VEGETATED	STONY	SANDY
a. Density: (No./20 l)			
June	2940	2921	1625
July	793	666	308
August	173	205	-
b. Total No. of taxa:			
June	9	8	8
July	9	7	9
August	8	9	-
c. Diversity:			
June	0.18	0.18	0.22
July	0.32	0.34	0.75
August	1.39	1.39	-
d. Evenness:			
June	0.08	0.09	0.11
July	0.15	0.18	0.34
August	0.67	0.63	-

Table 5.5: Characteristics of the benthos on vegetated, stony, and sandy substrates from Loch Lomond during June, July, and August 1985.

DATE	SUBSTRATE TYPE		
	VEGETATED	STONY	SANDY
a. Density: (No./m^2)			
June	3795	1612	780
July	3096	2500	850
August	5157	2856	-
b. Total No. of taxa:			
June	27	22	16
July	27	25	15
August	26	28	-
c. Diversity:			
June	2.45	2.29	1.63
July	2.01	2.12	1.63
August	1.89	2.09	-
d. Evenness:			
June	0.74	0.74	0.59
July	0.64	0.66	0.60
August	0.58	0.61	-

Table 5.6: Number of zooplankton and benthos sampled during June–August 1985 (rearranged from Table 5.3 to highlight the time effect). Note: the figures indicate number of zooplankton per 20 l water or number of benthos per sample, i.e. per 0.312, 0.25, and 0.187 m^2 for vegetation, stones, and sand respectively. (* = P <0.05; ** = P <0.01; *** = P <0.001; NS = not significant; X^2-tests).

PREY TYPES	VEGETATION			STONE			SAND	
	JUNE	JULY	AUGUST	JUNE	JULY	AUGUST	JUNE	JULY
ZOOPLANKTON								
Chydoridae	7	5	65 ***	2	6	49 ***	5	12 NS
Bosmina coregoni	2850	28	60 ***	2820	17	96 ***	1560	41 ***
Daphnia sp.	12	5	1 **	2	1	1	5	1
Polyphemida	5	4	2	1	1	10	14	6 NS
Diaptomus spp.	22	745	34 ***	35	624	45 ***	30	244 ***
Cyclops spp.	44	6	11 ***	61	17	4 ***	11	4 NS
BENTHOS								
Caenis sp.	107	15	0 ***	30	7	0 ***	3	0
Ephemerella ignita	54	10	0 ***	10	17	1 **	2	0
Ephemera danica	7	27	2 ***	2	0	4	0	0
Other Ephemeroptera	4	9	13 NS	5	2	5	0	1
Leuctra hippopus	31	16	102 ***	36	33	57 *	1	6
Coleoptera adult	48	16	20 ***	22	24	37 NS	0	3
Coleoptera larvae	169	45	93 ***	69	87	63 NS	4	3
Asellus aquaticus	23	8	16 *	13	32	11 ***	4	1
Chironomid larvae	128	75	743 ***	38	187	241 ***	15	22 NS
Chironomid pupae	27	7	56 ***	3	6	24 ***	2	1
Ceratopogonoid larvae	126	76	67 ***	14	36	12 ***	76	51 *
Oligochaeta	357	553	409 ***	128	157	205 ***	23	63 ***
Glassiphoniidae	32	44	28 NS	4	9	12 NS	1	0
Arachnida	11	7	12 NS	4	4	7 NS	1	1
Mulscus	1	13	3 ***	0	1	2	7	6 NS
Caddis larvae	34	20	1 ***	8	7	1 NS	7	1
Eurycercus lamellatus	25	25	44 *	17	16	32 *	0	0

5.3.4 Discussion

a. Substrate differences

In order to ascertain precisely the presence of prey species with a particular type of substrate, detailed studies of the ecological requirements of the species as well as the ability of the substrate to satisfy such requirments are needed. However, even though such studies have not been undertaken here, some associations have been noted between prey types and both substrate type and the time of the year.

The densities of zooplankton above vegetation and stones were similar and both were higher than that above sand, whereas the density of benthos decreased from vegetation to stones and sand. Diversity and evenness indices of zooplankton increased from vegetation and stones to sand, whereas diversity and evenness indices of benthos decreased. Moreover, the number of benthic taxa was higher on both vegetation and stones than on sand. The lower diversity and evenness indices of zooplankton on vegetation and stone compared to sand are due to the higher predominance of just 2 prey taxa (B. coregoni during June and Diaptomus spp. during July) above vegetation and stones. On the other hand, the greater diversity and evenness of benthic taxa on vegetation and stone compared to sand are a result of occurrence of more, and more abundant, taxa. As in the present study, Heck and Wetstone (1977) have found that above-ground vegetation biomass positively correlates with both prey abundance and richness.

Since the habitat in which an organism most commonly occurs is considered as the most favourable habitat (Threlkeld 1980), vegetated and stony habitats, rather than sandy habitat, seem to be good habitats for many zooplankton prey and for the vast majority of benthic prey recorded in this study as well. This may be due to the increased complexity (by means of increased vegetation cover, silt and refuges) of vegetated and stony substrates, which offers better food availability, living space, or protection from predators (Coen et al. 1981).

Many benthic prey (eg. Glassiphoniidae, E. ignita, Plecoptera) occurred in low density above sand (less than 4 individuals per sample). It is possible therefore that such occurrences are a result of species migration from the neigbouring substrates due to the high density, since many species are known to do so.

b. Monthly differences

The density of zooplankton decreased from June to August whereas the density of benthos increased. This was associated with an increase in the diversity and evenness indices of zooplankton and a decrease in the same parameters of benthos (on vegetation and stones but not on sand). The decrease in the density of zooplankton was a result of a reduction in the abundance of B. coregoni whereas the increase in the density of benthos was due mainly to the increase in the abundance of Chironomid larvae and pupae, Oligochaeta, and Plecoptera, although some other prey (Caenis sp., E. ignita, and caddis larvae) decreased or disappeared from the sample.

The increase and decrease in abundance of many invertebrate prey as summer progressed are probably due to recruitments of young individuals and progression of their life cycles (emergence or death) respectively. Seasonal changes in the physical factors that the invertebrate experience may play an important role in the variation in community structure (Watling 1975). This might be the reason for the higher variability in the benthos above vegetation and stone compared to above sand (which showed almost constant diversity and evenness indices); the physical structure of vegetated and stony substrates being more changeable due to changes in their vegetation cover.

Thus, sticklebacks foraging in areas with vegetation, stones, and sand encounter feeding patches with very different patterns of food availablility. Marked differences are found even within a few yards, from one habitat to another of different physical complexity, and from month to month within a single season.

5.4 PREY SELECTION BY THREE-SPINED STICKLEBACKS

5.4.1 Material and Methods

The fish

Three-spined sticklebacks (38-41 mm; standard length) used in this test were caught in Loch Lomond during the summer of 1985. Due to the shortage of sticklebacks in Loch Lomond during August, 50% of the fish tested at that time were caught in Balmaha Pond. The diets of the

fish from these two sites were broadly similar when tested in Loch Lomond where the density of zooplankton was high (see also Table 6.6, and Table 7.1). The fish were kept in the laboratory (13-16^{o}C) for 2 weeks prior to the test and fed daily on Tubifex worms.

The feeding enclosure

Field experiments were conducted in bottomless cylindrical enclosures (1 m in diameter) made of transparent nylon mesh (3 mesh/cm^2). This mesh retains the fish and allows movement of zooplankton in and out of the enclosures. The bottom edge of each enclosure was shaped with a metal frame on which a cloth tube containing some sand was attached. This cloth tube took the shape of the substrate on which the enclosure stood and prevented the fish escaping from underneath the frame. A floating plastic tube was attached on the top edge of each enclosure to prevent it from sinking in the water.

The substrates and test programme

Feeding tests were initiated just after sampling both benthos and zooplankton. Therefore, June and July tests were carried out on the three substrates mentioned above (Vegetation, stones and sand) but the August test included only vegetated and stony substrates because the fauna of the sandy substrate was not sampled during August (see above).

Test protocol

Experimental fish (deprived of food for 24 h to evacuate their stomachs, Beukema 1968) were introduced into the enclosures in batches of 3 fish at a time and left to feed on the available zooplankton and benthos. After 45 min the test was terminated and the fish were caught with a hand net, killed immediately and preserved for the subsequent analysis of stomach contents. The enclosures were then moved to new places in preparation for the next run of the test. A period of 45min was long enough for the fish to obtain moderately full stomachs (50-75% fullness) but not sufficiently long for either significant depletion of the food available or digestion of food in the stomachs. The locations of the enclosures were distributed over each substrate, and subjected to the condition that no enclosure was located twice in the same place. Overall, 24, 18 & 12 fish during June and 30, 24 & 15 fish during July were tested on vegetation, stone and sand respectively. During August, 39 and 32 fish were tested on vegetation and stones respectively.

Stomach content analysis

The food items in each stomach were identified to the lowest possible taxon, counted and weighed. The mean individual weight (wet weight after removing the excess alcohol) of each prey type was then calculated and proportioned to the mean individual weight of copepods (*Diaptomus* or *Cyclops*). This procedure was applied in order to avoid dealing with tiny-fraction numbers (the weights of the small prey organisms). A single copepod was therefore scored 1 unit and a two-times heavier individual was scored 2 units, and so on. After some of the stomachs in a given sample had been analysed in this way, it

became obvious that the relative weights of zooplankton prey types were more or less constant; therefore the proportional weights of such prey were applied for the rest of the stomachs. The proportional weight of a given food type was then multiplied by the total number of individuals of that food type in the stomachs to obtain the overall bulk units. The bulk of unweighable materials such as algae, plant tissues and sand particles was determined by eye.

The importance of each food type in the diet was ultimately expressed in terms of:

a. Bulk method: the number of units of a given food type in all stomachs examined was expressed as a percentage of the total units of all food types in the diet.
b. Numerical method: the number of items of a given food type found in all stomachs examined was expressed as a percentage of all food items in the diet. The uncountable materials (algae, plant tissues and sand particles) in the diet were not considered by this method.
c. Occurrence method: the number of stomachs in which at least one prey item of a given food type occurred was expressed as a percentage of the total number of occurrences of all food types. This method of expressing the percentage composition of occurrences was used rather than the usual method of expressing it as a percentage of stomachs that contained that food type. This facilitated comparison between the three methods as all added up to 100%, whereas the usual occurrence method does not necessarily add up to 100%.

The occurrence method of expressing the composition of the food in fish bears little indication to the actual amount of food eaten from a given food category. The numerical method over-estimates the

importance of numerous small items in the stomachs and cannot deal effectively with uncountable materials (e.g. algae). The volumetric method, however, has the opposite bias of over-estimating the importance of larger, yet less frequently eaten food items. Therefore, a combination of the three methods (percentage composition by bulk, by number and by occurrence) was used to gain the maximum information about the diet (Windell 1968, Hyslop 1980). A combination of both percentage by occurrence and either percentage by bulk or percentage by number for one sample gives an indication of the homogenity of feeding within the population (Hyslop 1980). For example, low percentage of occurrence and high percentage of number of a given food type indicate that only few fish from the population utilized this type of food and vice versa.

5.4.2 Data analysis

The results of the three methods of quantifying the diet were compared for each sample using Spearman's Rank Correlation Coefficient (Elliott 1983). Algae, plant tissues and sand particles were not included in the comparison since such materials could not be quantified by the numerical method. Food bulk diversity indices (H; Shannon and weaver 1949) and evenness indices (J; Pielou 1975) were calculated (see above) for each sample in order to study the differences in diet characteristics due to substrate and time differences. More specific statistical analyses were carried out only on the most common food types (see below); the percentage composition by bulk of a given common food-type was calculated for each stomach and a Mann-Whitney U-test was then used to test the

differences in the bulk consumed by fish feeding above various substrates and during various months.

The electivity indices of the common prey types in the diet were calculated for each sample using the relativized electivity index (E^*) of Vanderploeg & Scavia (1979):

$$E^* = [W_i-(1/n)] \; / \; [W_i+(1/n)]$$

where:

W_i= Chesson's (1978) alpha Coefficient = $(r_i/p_i) / \sum (r_i/p_i)$.
r_i= the proportion of food type i in the diet.
p_i= the proportion of food type i in the environment.
n= the number of food types available in the environment.

This index was used for its superiority in several aspects to the previously proposed electivity indices (Lechowicz 1982). For example, unlike Ivlev's (1961) electivity index, it allows meaningful comparisons of electivity values from samples with different relative abundance of their components. In addition, it takes into account the number of food types available in the environment and thus the electivity value of the food is a function of its abundance and the availability of other food types.

The index compares the proportion of a given food type in the stomachs with that available in the habitat and gives an indication of food types which are over-represented, under-represented, or eaten at random. The E^* values have a possible range between +1 and -1 for positive and negative selections respectively. An E^* value of 0 indicates absence of selection, i.e. the food type was eaten in a proportion similar to that in the environmental sample.

Because the index is sensitive for the food types which are rarely eaten (Lechowicz 1982), the electivity values of food types which

formed less than 1 % of the diet were not calculated. In addition, to reduce the effect of any possible sampling error, absence of electivity was arbitrarily set at values ranging from -0.1 to +0.1 (instead of 0), and all values above +0.1 indicated positive selection whereas those values below -0.1 indicated negative selection (Tompkins and Gee 1982).

Electivity values were calculated on the basis of bulk data (Cock 1978) of the food in the stomachs and in the environment. Because the potential food of sticklebacks consisted of both benthos (ultimately estimated as a number/m^2) and zooplankton (estimated as a number/20 l water; see above), it was necessary to standardize the two estimates of food types into a common scale. The number of zooplankton held above a square metre of a substrate was therefore calculated (this was possible since the water depth above the substrates was known, see above). Following this, the bulk of each food type from benthos and zooplankton found on or above a m^2 was calculated using the same method used in calculating the bulk of the food in the stomachs (Pages 130-131). Fish eggs, plant tissues and sand particles were not included in the calculations because the availability of such materials could not be assessed.

5.4.3 Results

The results of the stomach contents analyses are presented in Tables 5.7, 5.8, and 5.9 as percentage compositions by bulk, by number and by occurence respectively. The characteristics of the food eaten by the fish are presented in Table 5.10.

The three methods of quantifying the stomach contents were highly correlated (Table 5.11). Therefore, description of the diet was made only on the basis of data obtained from the bulk method. This method probably best highlights the relative importance of various food types in the diet (Ball 1961) and deals effectively with uncountable material such as algae and plant tissues.

Prey types consumed

The diet of three-spined sticklebacks consisted mainly of zooplankton, particularly Cladocera and Copepoda. Seven cladoceran and two copepod taxa were identified from the stomachs of the fish. B. coregoni (Cladocera) and Diaptomus spp. & Cyclops spp. (Copepoda) were the most important food for sticklebacks. Of the available benthos, Chironomid larvae & pupae, subimagines and E. lamellatus were of some importance during July and August. Other food types such as A. aquaticus, Corixid nymphs, Ceratopogonid larvae, water mites, Ephemeroptera, Plecoptera, flat worms, Ostracoda, and fish eggs were of minor importance. Traces of algal filaments, plant tissues and sand particles were also found in the stomachs. Terrestrial organisms, such as spiders, also occurred in the stomachs of sticklebacks.

Diet differences and substrate

During June, sticklebacks feeding above the three substrates ate B. coregoni, which formed the major food type, although this food type was consumed above sand more heavily than either above vegetation or stones (the difference was significant only between sand and stones,

Table 5.12). Cyclops spp. formed a considerable bulk of the diet of fish that fed above stones, but not in the diet of those that fed above either vegetation or sand. Daphnia sp. made a noticeable contribution to the food bulk only in the case of fish that fed above vegetation. Fish feeding above vegetation and stones included more types of food in their diet, with higher diversity and evenness indices than those that fed above sandy substrate.

During July, B. coregoni was still the major food in the diet of fish that fed above vegetation but not in the diet of those that fed above either stone or sand (this difference was significant in all comparisons, Table 5.12) where Diaptomus spp. were the major food type. In addition to Diaptomus spp. and B. coregoni, fish that fed above sand took considerable amounts of Chironomid larvae and of P. pediculus. Thus, even though the diet of fish that fed above sand contained fewer food types overall, it was characterized by higher diversity and evenness indices than the diet of fish that fed above vegetation and stones.

During August, fish feeding above vegetation relied on Diaptomus spp. to a great extent, although the amount eaten by fisht that fed above stones is still considerably high (no significant difference was found between the amounts eaten above these two substrates, Table 5.12). B. coregoni, E. lamellatus, Chironomid larvae and Chironomid pupae still made some contribution to the diet of fish that fed above vegetation. Fish feeding above stones mainly took E. lamellatus; although Cyclops spp. and Chironomid pupae, in addition to Diaptomus spp., still contributed noticeably to the diet. Thus, fish feeding above vegetation and stones relied on a wide range of food types and had almost similar food diversity and evenness indices.

Monthly variation in diet

The diet of three-spined sticklebacks underwent marked monthly changes in terms of the relative importance of food types included. Fish feeding above the three substrates reduced the amounts of B. coregoni in their diet as the time progressed towards August (this reduction was significant in all cases except from June to July, above vegetation, and from July to August, above stones, Table 5.13). This decrease in the amount of B. coregoni eaten generally coincided with an increase in the importance of Diaptomus spp. (this increase was significant from June to August and July to August, above vegetation, and from June to August, above stones, Table 5.13), Chironomid larvae, Chironomid pupae and E. lamellatus. Thus, as the time progressed towards August, the number of food types included in the diet and the food diversity and evenness indices increased .

Table 5.7: Percentage composition by bulk of the food eaten by three-spined sticklebacks feeding above different substrates during the period June-August 1985. T = Trace = <1% of the diet.

FOOD ITEMS	VEGETATION			STONE			SAND	
	JUNE	JULY	AUGUST	JUNE	JULY	AUGUST	JUNE	JULY
ZOOPLANKTON								
Sididae				T	T	1.2		
Chydorus sp.	T		T	T		T	T	T
Alona sp.	T	1.2	T	T	1.1	T	T	5.7
Bosmina coregoni	80.4	66.8	17.0	68.5	5.5	6.6	87.1	15.1
Daphnia sp.	9.7	T	2.1	1.2	1.3	1.6		6.8
Polyphemus pediculus	1.2	T	T	1.1			1.9	11.1
Bythotrephus longimanus	T	T	T		1.6			
Diaptomus spp.	T	6.1	35.1	1.4	54.6	19.7	3.8	39.7
Cyclops spp.	5.1	T	3.8	22.8	8.4	10.7	T	3.8
BENTHOS								
Caenis sp.				T				
Centroptilum luteolum						T		
Nemora erratica								T
Corixid nymph		T	1.8					1.2
Asellus aquaticus	T		T					
Chironomid larvae	T	4.9	10.5	T	6.7	5.6		14.9
Chironomid pupae	T	2.7	9.3		1.7	10.1		
Ceratopogonoidae			T			T		T
Subimagines	T	4.6	T	T	5.1		T	
Flat worm					4.1			
Water mite			T			T		
Spiders	T				T	T		
Eurycercus lamellatus	T	1.9	12.4	T	1.7	40.9		
Ostracoda	T							
Fish eggs		7.6					1.7	
Algae	T	T	T	T	T	T	T	T
Plant tissues	T	T	T	T	4.4	1.6	T	T
sand particles	T	T	T	T	3.4	T	4.2	T

Table 5.8: Percentage composition by number of the food eaten by three-spined sticklebacks feeding above different substrates during the period June to August 1985: T= Trace = < 1% of the food.

FOOD ITEMS	VEGETATION			STONE			SAND	
	JUNE	JULY	AUGUST	JUNE	JULY	AUGUST	JUNE	JULY
ZOOPLANKTON								
Sididae				T	T	T		
Chydorus sp.	T		T	T		T	T	T
Alona sp.	T	1.1	T	T	1.7	1.7	T	7.2
Bosmina coregoni	93.8	91.4	33.7	83.6	12.7	25.3	96.8	32.3
Daphnia sp.	1.9	T	T	T	T	T		1.7
Polyphemus pediculus	T	T	T	T			T	7.9
Bythotrephus longimanus	T	T	T		T			
Diaptomus spp.	T	4.2	38.7	T	68.6	36.9	2.1	42.3
Cyclops spp.	3.0	T	3.7	13.7	10.6	19.9	T	3.6
BENTHOS								
Caenis sp.				T				
Centroptilum luteolum						T		
Nemora erratica								T
Corixid nymph		T	T					T
Asellus aquaticus	T		T					
Chironomid larvae	T	T	1.5	T	2.9	2.3		4.2
Chironomid pupae	T	T	T		T	1.9		
Ceratopogonoid larvae			T			T		T
Subimagines	T	T	T	T	T		T	
Flat worm					T			
Water mite			T			T		
Spiders	T				T	T		
Eurycercus lamellatus	T	T	1.7	T	T	10.0		
Ostracoda	T							
Fish eggs		T					T	

Table 5.9 *Percentage composition by occurence of the food eaten by three-spined sticklebacks feeding above different substrates during the period June to August 1985.*

FOOD ITEMS	VEGETATION			STONE			SAND	
	JUNE	JULY	AUGUST	JUNE	JULY	AUGUST	JUNE	JULY
ZOOPLANKTON								
Sididae				1.7	2.4	3.6		
Chydorus sp.	0.9		0.6	3.4		0.9	3.0	1.6
Alona sp.	6.5	10.4	5.2	15.5	7.1	6.2	15.1	12.9
Bosmina coregoni	22.4	20.9	11.7	20.6	11.9	8.9	27.3	14.5
Daphnia sp.	14.0	3.4	6.5	5.2	4.7	1.8		9.7
Polyphemus pediculus	6.5	6.9	0.6	5.2			12.3	9.7
Bythotrephus longimans	2.8	2.3	1.2		2.4			
Diaptomus spp.	3.7	8.2	14.3	5.2	9.5	12.7	12.1	9.7
Cyclops spp.	14.0	3.5	7.1	15.5	7.1	12.7	6.0	8.1
BENTHOS								
Caenis sp.				3.5				
Centroptilum luteolum						0.9		
Nemora erratica								1.61
Corixid nymph		1.2	2.6					1.6
Asellus aquaticus	0.9		0.6					
Chironomid larvae	0.9	15.1	13.6	3.5	2.4	10.8		14.5
Chironomid pupae	1.9	2.3	8.4		2.4	7.2		
Ceratopogonoidae			0.6			0.9		1.6
Subimagines	2.8	2.3	1.3	1.7	4.8		3.0	
Flat worm					2.4			
Water mite			1.3			0.9		
Spider	0.9				2.4	0.9		
Eurycercus lamellatus	2.8	4.6	6.5	3.4	2.4	11.7		
Ostracoda	0.9							
Fish eggs		2.3					3.0	
Algae	4.6	4.6	4.5	3.4	9.5	5.4	3.0	1.6
Plant tissues	4.7	6.9	9.7	3.4	11.9	7.2	6.1	6.4
sand particles	8.4	4.6	3.3	8.6	14.1	7.2	9.1	6.4

Table 5.10: Characteristics of the food eaten by three-spined sticklebacks feeding above vegetation, stones and sand during the period June to August 1985.

DATE	SUBSTRATE TYPE		
	VEGETATED	STONY	SANDY
a. Total No. of food types in the diet			
June	15	12	8
July	13	13	11
August	16	14	-
b. Diversity:			
June	0.71	0.97	0.42
July	1.33	1.54	1.74
August	1.82	1.78	-
c. Evenness:			
June	0.26	0.40	0.20
July	0.52	0.60	0.73
August	0.65	0.67	-

Table 5.11: The statistics of Spearman's Rank test for the correlation between the percentages of bulk, number and occurrence of the food eaten by three-spined sticklebacks above different substrates and during different months. (* = P <0.05; ** = P <0.01; *** = P <0.001; NS = not significant).

COMPARISONS	VEGETATION			STONE			SAND	
	JUNE	JULY	AUGUST	JUNE	JULY	AUGUST	JUNE	JULY
BULK V NUMBER	0.84 ***	0.69 **	0.81 ***	0.77 **	0.68 **	0.81 ***	0.77 *	0.87 ***
NUMBER V OCCURRENCE	0.99 ***	0.78 **	0.95 ***	0.95 ***	0.82 ***	0.97 ***	0.86 **	0.80 **
OCCURRENCE V BULK	0.86 ***	0.40 NS	0.78 ***	0.76 **	0.45 NS	0.84 ***	0.50 NS	0.84 **
NUMBER OF PAIRS	15	13	16	12	13	14	08	11

Table 5.12: Summary of results showing the relationships between the amounts of Bosmina or Diaptomus eaten by three-spined sticklebacks above various substrates. (* = P <0.05, ** = P <0.01, *** = P <0.001, NS = not significant; U-test).

MONTH	SUBSTRATE	Bosmina			Diaptomus		
JUNE	Vegetation		NS			NS	
	Stones	NS		NS	NS		NS
	Sand	**			NS		
JULY	Vegetation		***			NS	
	Stones	***		**	NS		NS
	Sand	*			NS		
AUGUST	Vegetation		NS			NS	
	Stones	NS			NS		

Table 5.13: Summary of results showing the relationships between the amounts of Bosmina or Diaptomus eaten by three-spined sticklebacks during various months. (* = P <0.05, ** = P <0.01, *** = P <0.001, NS = not significant; U-test).

SUBSTRATE	MONTH	Bosmina			Diaptomus		
VEGETATION	June		NS			NS	
	July			***			***
	August		***			**	
STONES	June		***			NS	
	July			***			*
	August		NS			NS	
SAND	June						
	July		***			NS	

Prey selection by three-spined sticklebacks

Electivity indices for the most common food types in the diet of sticklebacks are presented in Table 5.14. In general, B. coregoni, Daphnia sp., Cyclops spp., P. pediculus (zooplankton) and Chironomid pupae (benthos) were positively selected by three-spined sticklebacks, whereas Chironomid larvae and Diaptomus spp. were negatively selected. The fish showed no general trend of selection for either Alona sp. or E. lamellatus. The vast majority of benthic prey were completely absent from the diet.

However, this trend of variation is not without exceptions, depending on the substrate and time differences. B. coregoni were positively selected above both vegetation and sand but not above stones (except during July). Chironomid larvae were positively selected only above sand (July). Diaptomus spp. were, in general, negatively selected, but were positively selected during August. Moreover, E. lamellatus were rarely eaten during June, negatively selected during July (vegetation and stones), and positively selected during August.

Table 5.14: Electivity indices for the most common food items consumed by three-spined sticklebacks feeding above different substrates during different months. -, 0, and + indicate negative, random, and positive selection respectively.

FOOD ITEMS	VEGETATION			STONE			SAND	
	JUNE	JULY	AUGUST	JUNE	JULY	AUGUST	JUNE	JULY
ZOOPLANKTON								
Sididae						+		
Alona sp.		-			0			+
Bosmina coregoni	+	+	+	-	+	0	+	+
Daphnia sp.	+		+	+	+	+	-	+
Polyphemus pediculus	+			+		-	+	+
Bythotrephes longimanus					+	-		
Diaptomus spp.		-	+	-	-	+	0	-
Cyclops spp.	+		+	+	+	+		+
BENTHOS								
Corixidnymph	-		0			-		+
Chironomid larvae		-	-		-	-	-	+
Chironomid pupae		+	+		+	+		
Subimagines		+			+	-		-
Eurycercus lamellatus		-	+		-	+		

5.4.4 Discussion

Diet composition

The diet of the three-spined sticklebacks used in this study consists mainly of zooplankton; benthos usually is of little importance despite its high abundance in the habitat. Algae, plant tissues and sand particles have been occasionally recorded in the diet, as it is the case in other studies on sticklebacks (eg. Manzer 1976, Ukegbu 1986). Such material may have been passively consumed while consuming other prey items.

It is likely that the microhabitat choice and predator avoidance of the prey affect their vulnerability to predation. Zooplankton, which inhabit the water body, are probably more accessible than benthos and consequently are more susceptible to predation by sticklebacks. Benthos, which inhabit complex environments of the lake bed, are probably less frequently detected by potential fish predators (Ware 1973, Anderson 1984). Thus, the under-representation of many benthic prey types in the diet may not be due to a true rejection of these prey by the fish but may be a result of failure to encounter them. Benthos, however, becomes more important as the density of zooplankton decreases, with fish becoming more dependant on Chironomid larvae, Chironomid pupae, and E. lamellatus during August compared to June and July.

Capture of a given prey type was influenced not only by its abundance in a given habitat but also by the type of substrate above which it occurred. B. coregoni was dominant in the diet of fish feeding above the three substrates during June when its density was

also high in the habitats. Although its density decreased sharply during July, B. coregoni was still heavily consumed by sticklebacks feeding above vegetation and not by those feeding above either stone or sand, even though its density above sand was higher than that above vegetation. At the same time, Diaptomus spp. (which dominated the zooplankton above the three substrates during July) contributed little to the diet above vegetation but was still heavily consumed above stone and sand. Similarly, in August, E. lamellatus were heavily consumed above stones but not above vegetation, despite their higher availability above the latter substrate.

Although sticklebacks consumed a wide variety of food types in their diet, they showed a positive selection for particular types and apparently avoided others. B. coregoni, Cyclops spp., Daphnia sp. and P. pediculus among zooplankton as well as Chironomid pupae among benthos were the most preferred food types. In contrast, Diaptomus spp. and Chironomid larvae were negatively selected.

Thus, zooplankton is the predominant and the most preferred food (as indicated by the electivity indices) of three-spined sticklebacks and as the density of such food decreases the importance of the benthos in the diet increases. Moreover, the changes in the abundance of zooplankton, such as Diaptomus, result in similar changes in their representation in the diet of sticklebacks feeding above stone and sand but not above vegetation, reflecting the effect of substrate type on food consumption.

Functional interpretations

Sticklebacks feed predominantly on zooplankton rather than benthos. Zooplankton give a higher rate of energetic return (i.e. more profitable) than benthos (Chapter 3). Thus, the behaviour of sticklebacks in this case is in accordance with the prediction of Optimal Foraging Theory.

Optimal Foraging Theory predicts that animals should broaden their diet to include more food types as prey density decreases, i.e. the diversity and evenness indices of the food types in the diet should increase (Schluter 1981, Horn 1983). Despite the lower density of zooplankton above sand during June compared to above vegetation and stones, sticklebacks feeding above sand did not broaden their diet; food diversity and evenness indices above sand were lower than those above vegetation and stone. This may be because the density of B. coregoni (the preferred prey species, see above) above sand was still sufficiently high to be worth the sticklebacks to concentrate feeding on. By July, when the density of B. coregoni above sand (and above vegetation and stones) declined further (which results in a decrease in the overall zooplankton density), sticklebacks feeding above sand broadened their diet and ingested food of higher diversity and evenness of its components compared to those feeding above vegetation and stones. Furthermore, by August, when zooplankton density continued to decline above both vegetation and stones, sticklebacks broadened their diet and ate more benthos (Chironomid larvae and pupae, and E. lamellatus), thus their diet characterized by higher diversity and evenness indices. This behaviour was therefore in accordance with the prediction of Optimal Foraging Theory.

Causal interpretations

Considering just the actual food eaten by three-spined sticklebacks, zooplankton and only the most active prey of benthos (Chironomid larvae, Chironomid pupae, subimagines and E. lamellatus) were prominently eaten. Feeding on active prey has been shown in other fish such as rainbow trout, Salmo gairdneri (Ware 1973). Despite their distinctive movement pattern and their availability in the habitat, water mites were rarely eaten. The most likely explanation for this observation is that such invertebrates produce noxious chemicals which are unpalatable to the fish (Hyatt 1979). Sticklebacks have taste receptors in their mouth (Wootton 1976) and they respond actively to the palatability of food (Beukema 1968, Thomas 1974).

The high consumption of B. coregoni above vegetation and Diaptomus spp. above stones and sand (during July) may be directly linked to their contrast against these substrates. Bosmina coregoni, with the faded-colour and black eye spots, might appear more conspicuous against the dark green vegetation while Diaptomus spp., which are red, might appear more conspicuous against the light coloured sand or stones.

Considering food selection, sticklebacks positively select the prey items which possess one or more preferred visual properties. For example, the predominant positive selection for zooplankton can be explained by the fact that such prey, in addition to the simplicilty of their habitat, possess many visual properties which are preferred to sticklebacks. Compared to benthic prey, they have distinctive movement-pattern and the majority of copepods have red colour. Fast movement (up to a certain limit) and red colour of the prey were found

to be important in diet choice in sticklebacks (Chapter 4). The observed positive preference for Chironomid pupae among other benthic prey supports the importance of movement in food selection by sticklebacks, since such organisms increase their movement before emergence.

The observed positive selection for B. coregoni, Daphnia sp. and P. pediculus of Cladocera rather than Alona sp. may be due to their conspicuous black compound eyes against transparent bodies. This feature is known to increase the reactive distance of the prey (Ware 1971, Zaret 1972), and selectivity by fish (Zaret and Kerfoot 1975).

Among copepods (which are very similar in general appearance but not by movement), Cyclops spp. were selected more strongly than Diaptomus spp. even when the latter prey were more abundant. It is possible that the less evasive movement (which increases the feeding efficiency of the fish) of Cyclops spp., compared to that of Diaptomus spp. (O'Brien 1979), is the reason behind the over-representation of such prey in the diet. However, Diaptomus spp. were positively selected only during August when the availability of other zooplankton was at the minimum.

Although Chironomid larvae possess many preferred visual cues for sticklebacks (Chapter 4), they were under-represented in the diet. Most of Chironomid larvae are case-dwellers and this might reduce their exposure to predators (Hershey 1987) and reduce the encounter rate. Consequently, Chironomid larvae might have been consumed only when they were outside their cases. Alternatively, the predominant feeding on zooplankton by sticklebacks might have restricted their search path to the water column, as a successful place for foraging, and consequently less encounter rate with benthic chironomids occurred.

5.5 PREY-SIZE SELECTION BY THREE-SPINED STICKLEBACKS

5.5.1 Material and Methods

Prey size selection was investigated in the fish tested during August 1985 on the vegetated substrate in Loch Lomond (see above). Length-frequency distribution of the prey in the stomachs was compared with length-frequency distribution of the prey sampled from the habitat during August 1985 (see above). This sample was chosen for such analysis because the sticklebacks that tested during August fed on a wider range of food types (see above) which can be included in the analysis. Prey individuals were measured (to the nearest 0.05 mm) as the distance from the tip of the head to the end of the abdomen (appendages were excluded from the length). Only prey types which are common in the diet were considered. When large number of individuals of a given prey type occurred, 50 individuals were randomly chosen and measured to represent the length distribution. The copepods Diaptomus and Cyclops are very similar in body size and were therefore treated as one food type.

5.5.2 Data analysis

The degree of size selection, S, of a given food type was measured using the index described by Bartell (1982):

$$S = (L_e - L_a) / L_r$$

where:

L_e = mean length of prey in the stomachs.
L_a = mean length of prey available in the habitat.
L_r = range of prey length in the pooled prey from the stomachs and the habitat.

The index gives possible values ranging from -1 to +1 for smaller and larger prey eaten respectively. A value of 0 indicates that no size selection occurred (i.e. random size selection). Prey items whose length deviated greatly from the mean were omitted from the data because the index is sensitive to such measurements (Bartell 1982). However, such items occurred rarely and only in the case of Chironomid larvae eaten.

5.5.3 Results

Size-selectivity values of the common food types and the mean sizes of prey found in the habitat and in the stomachs are presented in Table 5.15. The fish showed a tendency to consume larger individuals of zooplankton, B. coregoni & copepods, and smaller individuals of benthos, Chironomid larvae, Chironomid pupae, and E. lamellatus.

Table 5.15: Size-selectivity indices and mean length (mm ± S.E.) of the common food types in the habitat and in the stomachs of three-spined sticklebacks feeding during August 1985 on a vegetated substrate in Loch Lomond.

PREY TYPES	SIZE-SELECTIVITY INDICES	MEAN LENGTH	
		Habitat	Stomachs
ZOOPLANKTON:			
Bosmina coregoni	0.08	0.45 ± 0.03	0.48 ± 0.02
Copepods	0.11	1.03 ± 0.04	1.12 ± 0.03
BENTHOS:			
Chironomid larvae	-0.44	5.15 ± 0.71	2.12 ± 0.02
Chironomid pupae	-0.63	4.45 ± 0.54	2.01 ± 0.03
Eurycercus lamellatus	-0.30	2.60 ± 0.11	1.90 ± 0.06

5.5.4 Discussion

Three-spined sticklebacks showed a tendency to select larger size classes of zooplankton (B. coregoni and copepods) and smaller size classes of benthos (E. lamellatus, Chironomid larvae and Chironomid pupae). It may be that sticklebacks have a threshold prey size (approx. 1.02-2.61 mm, Table 5.15) under which they select the larger prey available and above which they select the smaller prey available.

The observed selection for larger zooplankton by sticklebacks is in accordance with the prediction of Optimal Foraging Theory since the energetic content of zooplankton increases with body size (Chapter 3) while handling time of these smaller prey remains more or less constant (Werner and Hall 1974, Mittelbach 1981, see also Chapter 3). However, the observed selection for the smaller individuals of benthos does not agree with the prediction of Optimal Foraging Theory, since sticklebacks are able to handle larger size classes (up to a certain limit) with higher energetic return (Chapter 3) and showed a preference towards such larger sizes (Chapter 4). In the experiments described in chapters 3 and 4, the prey were presented in unnatural ways where the body size of the prey was the sole problem involved in their discovery. In contrast, in natural habitats some factors other than size may be involved in prey selection. Larger prey individuals may be better at avoiding predators than smaller ones. Alternatively, three-spined sticklebacks may have sought to minimize handling time, by eating smaller benthic individuals, in favour of other activities required in the field such as predator avoidance (Chapter 7). Tufted ducks (Aythya fuligula) select suboptimal, smaller mussels (Dreissena polymorpha) even when larger, optimal mussels are available, possibly because taking the longer-to-handle optimal prey reduces the ability of birds to detect predators (Draulans 1984).

5.6 COMPARISON OF DIET OF THREE-SPINED AND TEN-SPINED STICKLEBACKS

5.6.1 Material and Methods

While testing three-spined sticklebacks for food selection above vegetation during August 1985 (see above), twenty eight ten-spined sticklebacks (32-36 mm, standard length) were tested on the same substrate for the same purpose. The diet of the two fish species can therefore be compared with each other and with the food available in the habitat at that time. Tests of three-spined sticklebacks and ten-spined sticklebacks were run simultaneously and in the same way described above (Page 130). Stomach contents of both fish groups were expressed as percentage composition by bulk and by occurrence (Pages 130-131).

5.6.2 Data analysis

Selectivity indices of the common food types were calculated using Vanderpleog and Scavia (1979) electivity index (Pages 133-134). To test the difference in the bulk of the common food types eaten by both fishes, the bulk of a given food type in each stomach was expressed as a percentage of the total bulk contained and a Mann-Whitney U-test was used to test the significance of the difference.

5.6.3 Results

Diet composition

Table 5.16 shows that the diet of three-spined sticklebacks consisted mainly of zooplankton, namely Diaptomus spp. and B. coregoni. Among benthos, E. lamellatus, Chironomid larvae and Chironomid pupae were of some importance. In contrast, the diet of ten-spined sticklebacks mainly consisted of benthos, namely Chironomid larvae and (to a lesser extent) Chironomid pupae and E. lamellatus. Zooplankton was of less importance; Cyclops spp. were the most important prey among zooplankton. Moreover, the stomachs of ten-spined sticklebacks contained plant tissues and sand particles more than those of three-spined sticklebacks. The difference between the diet of both fishes is significant in the case of B. coregoni and Diaptomus spp. but not in the case of Cyclops spp., Chironomid larvae, Chironomid pupae or E. lamellatus.

Food selectivity

As shown in Table 5.17, three-spined sticklebacks showed a positive selection for B. coregoni, Daphnia sp., Diaptomus spp. and Cyclops spp. among zooplankton as well as Chironomid pupae and E. lamellatus among benthos. In contrast, ten-spined sticklebacks showed a positive selection for Chironomid pupae, subimagines, gastropoda, and E. lamellatus) among benthos and only Cyclops spp. among zooplankton.

Table 5.16: Percentage composition by bulk and by occurrence of the food eaten by three-spined (rewritten from Tables 5.7 and 5.9 respectively for the comparison) and ten-spined sticklebacks feeding above vegetation during August 1985. T = Trace = < 1% of the bulk. * = P <0.05, ** = P <0.01, NS = not significant; U-test.

FOOD TYPES	% OCCURRENCE		% BULK		
	3-SPINED	10-SPINED	3-SPINED	10-SPINED	
ZOOPLANKTON					
Chydorus sp.	0.6	2.17	T	T	
Alona sp.	5.2	4.34	T	T	
Bosmina coregoni	11.7	4.34	17.0	1.2	*
Daphnia sp.	6.5	2.17	2.1	1.6	
Polyphemus pediculus	0.6		T		
Bythotrephus longimanus	1.2		T		
Diaptomus spp.	14.3	4.34	35.1	2.8	**
Cyclops spp.	7.1	6.50	3.8	14.3	NS
BENTHOS					
Corixid nymph	2.6		1.8		
Asellus aquaticus	0.6	2.17	T	4.7	
Chironomid larvae	13.6	24.0	10.5	30.0	NS
Chironomid pupae	8.4	4.34	9.3	16.3	NS
Ceratopogonoidae	0.6		T		
Subimagines	1.3	4.34	T	4.2	
Water mite	1.3		T		
Gastropoda		4.34		3.8	
Eurycercus lamellatus	6.5	10.8	12.4	16.0	NS
Algae	4.5	2.14	T	T	
Plant tissues	9.7	15.2	T	2.7	
Sand particles	3.3	8.69	T	1.4	

Table 5.17: Selectivity indices for the most common food types in the diet of three-spined and ten-spined sticklebacks feeding above vegetation during August 1985. (-, 0 and + indicate negative, random, and positive selection respectively).

FOOD TYPES	3-SPINED	10-SPINED
ZOOPLANKTON:		
Bosmina coregoni	+	-
Daphnia sp.	+	0
Diaptomus spp.	+	-
Cyclops spp.	+	+
BENTHOS:		
Corixid nymph	0	
Asellus aquaticus		-
Chironomid larvae	-	-
Chironomid pupae	+	+
Subimagines		+
Gastropoda		+
Eurycercus lamellatus	+	+

5.6.4 Discussion

The diets of three-spined and ten-spined sticklebacks are clearly different from each other, with the former fish relying on zooplankton and the latter fish relying on benthos. This has been shown both in terms of the amount of food consumed and the broad type of food they mostly prefer (as indicated by electivity indices).

It seems that the preferred microhabitat of both species of fish influences the food selection. Three-spined sticklebacks prefer open water areas and ten-spined sticklebacks prefer dense vegetation and

algal areas (Wootton 1984). Thus, both three-spined and ten-spined sticklebacks eat the food which is available in their respective preferred habitat.

Among copepods (zooplankton), ten-spined sticklebacks showed a positive selection only for Cyclops spp., which is less evasive than Diaptomus spp. whereas three-spined sticklebacks showed a positive selection for both Diaptomus spp. and Cyclops spp. This may be an inherited feature of Loch Lomond fish or may be because the full range of experience with zooplankton (see above) made them more efficient at catching the more evasive copepods (see Chapter 3).

The differences in the feeding habits of three-spined and ten-spined sticklebacks suggest that the feeding competition between the two fish species is unlikely to occur. Such competition, however, may be stronger in winter when food is in short supply, especially if each fish species broadens its diet (see above) to include the less preferred food, which is the diet of the other species.

5.7 CONCLUSIONS

1. The potential food of three-spined sticklebacks is variable in space and in time, with vegetated and stony substrates providing a wider range of prey types and higher density of both zooplankton and benthos than the sandy substrate. The density of zooplankton is high during June and decreases as summer progresses towards August whereas the density of benthos does the reverse.

2. Sticklebacks in Loch Lomond feed predominantly on zooplankton prey and disregard many available benthic prey. Comparison of the food eaten with that available in the habitat indicates that three-spined sticklebacks positively select zooplankton. Benthic prey become more important as zooplankton density declines. Food consumption by sticklebacks is influenced by the substrate type above which the food occurs and by monthly changes in food abundance.

3. Three-spined sticklebacks select prey items which are exposed, active but less evasive and red in colour. Thus, they are using simple proximate cues to select their food and these cues lead them to the most profitable food (zooplankton).

4. From the prey available in the habitats, sticklebacks select slightly-larger individuals of zooplankton and smaller individuals of benthos.

5. Food selection of three-spined sticklebacks (during summer) is unlikely to be affected by the coexistence with ten-spined sticklebacks, since these two species have distinctively different diets and show a positive selection for different prey types.

CHAPTER 6

MORPHOLOGY, FEEDING HABITS, AND HABITAT USE OF STICKLEBACKS FROM TWO SCOTTISH POPULATIONS

6.1 INTRODUCTION

6.1.1 Intraspecific variation in stickleback populations

In the previous chapters, various aspects of feeding behaviour of three-spined sticklebacks have been investigated. The present chapter focuses on the differences in behaviour and morphology between two stickleback populations (Loch Lomond and Balmaha Pond fish) and attempts to relate such differences to the environmental ecology of the fish.

As mentioned in chapter 1, extensive variations in a number of behavioural and morphological characteristics are found within the low-plated (leiurus) form of stickleback. Such variations were found between populations inhabiting lotic (eg. rivers, streams) and lentic (eg. lakes, ponds) habitats (Hagen and Gilbertson 1972, Coad and Power 1974, Gross and Anderson 1984), between populations inhabiting lakes with different ecological properties (Lavin and McPhail 1985, 1986) and between populations inhabiting different parts of a single lake (Larson 1976, Ridgeway and McPhail 1984, Bentzen and McPhail 1984).

In order to understand the significance of these variations, comparisons have been made between the morphological traits of stickleback populations and the ecological conditions under which they live. Such comparisons have revealed a clear interpopulation variability in morphology (Bell 1984) and many comparative studies (eg. Gross 1978, Lavin and McPhail 1986) have related this to differences either in diet, in predation pressure, or in both.

6.1.2 Behavioural and morphological differences that can be related to diet

Two distinctive biological forms of stickleback, limnetic and benthic, have been identified; the names alluding to their distribution within the habitat (Ridgeway and McPhail 1984). Limnetic sticklebacks inhabit the water body of deeper waters, whereas benthic sticklebacks inhabit lake-bottoms and weedy areas of littoral zones. Increasing vegetation cover causes limnetics to aggregate away from cover near the water surface and benthics to disperse among weed (Larson 1976).

Benthic sticklebacks are also associated with small ponds dominated by littoral regions, whereas limnetic sticklebacks are associated with large lakes dominated by limnetic regions (Lavin and McPhail 1986). Sticklebacks intermediate between limnetic and benthic forms have also been identified, and found to be associated with an intermediate size of lakes and carry the intermediate features of the other two forms.

The habitats of limnetic and benthic sticklebacks differ in the type of food available; the water body is dominated by small prey (zooplankton) whereas the lake bottom is dominated by larger prey (benthos). As a consequence, limnetic sticklebacks feed on the organisms that live in the water column (i.e. zooplankton), whereas benthic sticklebacks feed on organisms which live on the substrate, i.e. benthos (Larson 1976, Lavin and MacPhail 1985, 1986). However, the type of food eaten by limnetic and benthic sticklebacks may not necessarily be the preferred food; a food type may be taken simply because it is the only sufficiently available food. In this case, the difference in diet would, therefore, be a secondary effect of the shift in habitat use of both forms of stickleback.

In addition to variation in their habitats and diet, limnetic and benthic sticklebacks differ in the morphology and deployment of their feeding apparatus including gape size and gillraker spacing, number and length. Thus, benthic sticklebacks have wider jaws, allowing them to handle benthic prey of a given size within shorter time than limnetic sticklebacks (Bentzen and McPhail 1984). Moreover, limnetic sticklebacks possess closer, longer and more numerous gillrakers than benthic sticklebacks. Such characteristics increase the efficiency of limnetic and benthic sticklebacks when feeding on their respective food (Lavin and McPhail 1985, 1986). Closer, longer and more numerous gillrakers are thought to dictate the minimum prey size that can be extracted from the plankton, reducing the probability that a plankter will escape through the gills once in the mouth (Hyatt 1979, Zaret 1980, Wright et al. 1983). Thus, closer gillrakers would be expected to increase the efficiency of such fish when feeding on zooplankton.

6.1.3 Morphological differences that can be related to predation risk

Regardless of whether they are limnetic or benthic, stickleback populations may differ in a number of morphological features that may confer protection against predators. These differences appear in body size, dorsal and ventral spines length and number of lateral plates.

Body size may reflect local availability of profitable food resources (Miller 1979), level of predation pressure upon fish population, or both. Larger body size may enhance the ability of fish to avoid predation (Moodie 1972), with larger fish requiring

greater manipulation in the mouth of predators than small fish. Also, visual acuity (Baerends et al. 1960, cited in Moodie 1972) and swimming speed (Bainbridge 1958) are an increasing function of fish size and therefore larger fish should be able to detect approaching predators earlier and escape faster than smaller fish. Thus, body size is greater in stickleback populations in contact with predators.

Dorsal and ventral spines, when erected, rapidly increase the effective size of sticklebacks and form a triangular zone within which the fish body is unlikely to be damaged (Giles 1987 b). Thus, spine length may well correlate with the predation pressure upon stickleback populations (Hoogland et al. 1957, Gross 1978). The number of lateral plates in sticklebacks from North American populations also varies with predation risk; fish with 7 lateral plates on each side of the body dominate stickleback populations that are exposed to high risk of predation (Hagen and Gilbertson 1972, 1973). The reasons for this association are not clear and in addition, in European populations of stickleback, no correlation has been found between lateral plate number of 7 and level of predation risk (Gross 1977).

6.1.4 Loch Lomond and Balmaha Pond sticklebacks

The studies described in the previous sections give broad but necessary superficial surveys of morphological variation in stickleback populations in relation to diet and predation risk, and suggest that such variations represent adaptations to local ecological conditions (Gross 1978, Lavin and McPhail 1986). In order to

investigate further the nature of these adaptations and to explore certain behavioural differences described in the previous section, detailed morphological and behavioural comparisons were made between just two populations, Loch Lomond and Balmaha Pond, and the result interpreted against the broader survey described above.

It was shown in chapter 3 that sticklebacks from Balmaha Pond tended to be more efficient at feeding on benthos than fish from Loch Lomond. This finding suggests that Balmaha Pond fish, unlike Loch Lomond fish, may be adapted to feeding on benthos. In addition, casual observations on Balmaha Pond fish have shown that they have shorter spines and behave differently from Loch Lomond fish when disturbed by an observer. Balmaha Pond fish are very frightened at sighting the observer and tend to jump down and remain in contact with the substrate among the weed whereas Loch Lomond fish are slower at making such response and show no general trend of jumping down towards the substrate. Most published studies have shown that fish from low predation risk sites have poorly developed anti-predator responses compared to fish from high predation risk sites (Segher 1974, on guppies, Poecilia reticulata; Magurran 1986, on minnows, Phoxinus phoxinus, Giles and Huntingford 1984, on three-spined sticklebacks, G. aculeatus). Magurran (1986) has observed that minnows sympatric with pike (Esox lucius) are more timid and keep a greater distance from the predator than minnows derived from predators-free sites. In contrast, Fraser and Gilliam (1987) have observed that guppies sympatric with predators maintain their feeding longer than guppies from localites with no predators. This suggests that the behavioural differences between sticklebacks from the two populations under consideration (Loch Lomond and Balmaha Pond) may reflect differences in the levels of predation pressure they incur.

6.1.5 Aims

The specific aims of this work are:

1. To determine the differences between Loch Lomond and Balmaha Pond in terms of food availability and to investigate the differences between fish from both sites in terms of food selection and habitat use.

2. To investigate the differences between Loch Lomond and Balmaha Pond fish in the morphological traits that may be related to differences in feeding habit and in predation pressure.

6.2 DESCRIPTION OF THE STUDY SITES

The two sites studied (Loch Lomond and Balmaha Pond) differ markedly in a number of characteristics. Loch Lomond is much larger (71.1 km^2) than Balmaha Pond (0.001 Km^2). Loch Lomond is dominated by open areas and has high density of both zooplankton and benthos whereas Balmaha Pond is dominated by submergent and emergent vegetation and has a low density of zooplankton and a high density of benthos (Chapter 2; Pages 20-21).

Loch Lomond has a wide variety of both fish and birds predators whereas Balmaha Pond has no fish predators but may be visited by some

bird predators. However, the thick cover of vegetation in Balmaha Pond may reduce the predation pressure from birds upon its sticklebacks. The sampling station of Loch Lomond was a shallow water (40-50 cm deep) of Mill of Ross Bay (NS 368 968) which has a bed of thick submergent aquatic littoral flora (10 cm high). The sampling station of Balmaha Pond included the whole Pond except an area of c. 8 m in diameter in the center of the pond. This area had a deep muddy bottom which was difficult to sample with the equipment available.

6.3 DIET COMPOSITION IN THE NATURAL HABITATS

6.3.1 Material and Methods

Food available

Zooplankton and benthos available in the two study sites were quantitatively sampled. Five water samples (4 l each) and five core samples (30 cm in diameter) were taken randomly from each station as representative samples of zooplankton and benthos respectively. Water samples were collected by filtering the water through a standard zooplankton net. Core samples were taken by screwing the sampler into the substrate (with its top end remaining above the water surface) and dislodging the substrate inside (to a depth of 5 cm) until the soil and the accompanied invertebrates were suspended in the water. The water inside the sampler was then sieved by using a 400 μ sieve. Sieving was continued until 5 successive sieves

brought no single organism. Individuals of zooplankton and benthos were separated from the samples, identified (see the methods in chapter 5; Pages 113-115) and recorded as a number per 20 l water (sample volume) and per 0.35 m^2 (sample area) respectively.

Food eaten

Forty sticklebacks from Loch Lomond and 30 others from Balmaha Pond were caught on the morning (10 am, just before collecting samples of the food available) at the end of June 1986. They were killed immediately and preserved for a week before they were analysed. Stomach contents were removed, identified to the lowest possible taxon, and specified as belonging to one of these two general categories:

a. Zooplankton: included those organisms that inhabit the water column exclusively (see Table 6.3).
b. Benthos: included those organisms that inhabit the lake bed partially or totally (see Table 6.3).

After these identifications, the percentage composition by bulk and by occurrence of each taxon from zooplankton and benthos were calculated (see the methods in chapter 5, Pages 130-131). The percentages of overall bulk of zooplankton and benthos in each stomach were also calculated. The electivity values of the most common food types were calculated in the same way described in chapter 5 (Pages 133-134).

6.3.2 Data analysis

Food available and food eaten

A X^2-test was used to test the differences in the total numbers of zooplankton and benthos sampled from Loch Lomond and Balmaha Pond. A Mann-Whitney U-test was used to test the difference between Loch Lomond and Balmaha Pond fish in the percentage of zooplankton and benthos eaten.

6.3.3 Results

Food available

The data of zooplankton and benthos available in Loch Lomond and Balmaha Pond are presented in Table 6.1. Loch Lomond had higher density of zooplankton than Balmaha Pond. The predominant zooplankton were copepods in Loch Lomond and chydorids in Balmaha Pond. In contrast, Balmaha Pond had a greater density of benthos than Loch Lomond, even though the density of benthos in Loch Lomond was still high. In both sites the predominant benthos were Chironomid larvae and Oligochaeta.

Food eaten

As Table 6.2 shows, Loch Lomond fish consumed more zooplankton (the small prey sizes) than Balmah. pond fish (the larger prey sizes), whereas Balmaha Pond fish consumed more benthos than Loch Lomond fish. Considering diet composition (Table 6.3), the diet of Loch Lomond fish

consisted mainly of copepods (both by bulk and by occurrence). Chydorids, Bosmina coregoni, and Daphnia sp. occurred in a considerable number of the stomachs, but they contributed little to the total bulk. Of the available benthos, Chironomid larvae and pupae, Ceratopogonoid larvae, and fish eggs were found in small amounts in the stomachs of sticklebacks from Loch Lomond. The other food types listed in Table 6.3 occurred only in few stomachs and contributed little to the diet. The food of Balmaha Pond fish consisted mainly of Chironomid larvae (both by bulk and by occurrence). Chironomid pupae occurred in a considerable number of stomachs but in small amounts. The other benthic food types listed in Table 6.3 occurred in few stomachs and contributed little to the total bulk. Of the available zooplankton, copepods and chydorids were eaten by most of the fish and contributed considerably to the total bulk.

Algae, plant tissues, and sand particles were occasionally found in the stomachs of sticklebacks from both sites but more commonly in those from Balmaha Pond.

Electivity indices as a measure of food preference

The electivity indices of the common food types eaten by sticklebacks from Loch Lomond and Balmaha Pond are presented in Table 6.4. Among zooplankton, copepods were positively selected by both Loch Lomond and Balmaha Pond fish. Chydorids were positively selected by Balmaha Pond fish but were rarely eaten by Loch Lomond fish (i.e. their electivity indices were not calculated, see above). Among benthos, Chironomid pupae were positively selected by both Loch Lomond and Balmaha Pond fish. Subimagines were selected at random by Balmaha Pond fish but were rarely eaten by Loch Lomond fish.

Table 6.1: Number of zooplankton (per 20 l water) and benthos (per 0.35 m^2) available in Loch Lomond and Balmaha Pond during June 1986. (*** = P <0.001; X^2-test)

FOOD TYPE	LOCH LOMOND	BALMAHA POND	
ZOOPLANKTON:			
Copepods	158	5	
Chydorids	24	14	
Bosmina coregoni	36	2	
Daphnia sp.	3	0	
Nauplii	8	0	
TOTAL ZOOPLANKTON	229	21	***
BENTHOS:			
Ostracoda	1	1	
Chironomid larvae	56	350	
Chironomid pupae	2	7	
Ceratopogonoid larvae	23	15	
Oligochaeta	121	75	
Caddis larvae	30	7	
Insecta nymphs	19	4	
Subimagines	2	1	
Asellus aquaticus	6	0	
Gastropods	0	6	
TOTAL BENTHOS	260	466	***

Table 6.2: Median percentage b y bulk of zooplankton and benthos consumed by Loch Lomond and Balmaha Pond fish in their natural habitats. The approximate size (thickness and length) range of individual prey was also given for each food category.
(*** = P <0.001, U-test)

FOOD TYPE	APPROX. SIZE RANGE (mm)		MEDIAN PERCENTAGE FOOD BULK	
	thickness	length	Lomond fish	Balmaha fish
ZOOPLANKTON	0.15-2.00	0.15-3.5	94.75	19.25 ***
BENTHOS	0.30-2.50	1.5 - 30	5.25	80.75 ***

Table 6.3: Percentage composition by bulk (upper figure) and by occurrence (lower figure) of zooplankton and benthos eaten by Loch Lomond and Balmaha Pond fish in their natural habitats.

FOOD TYPE	LOMOND FISH n=40	BALMAHA FISH n=30
ZOOPLANKTON:		
Copepods	81.50 22.4	8.55 17.6
Chydorids	0.40 11.6	3.3 14.0
Bosmina coregoni	0.89 14.7	0 0
Daphnia sp.	0.82 7.0	0 0
BENTHOS:		
Ostracoda	0.11 1.5	0.71 7.7
Chironomid larvae	5.35 12.4	64.80 21.1
Chironomid pupae	1.53 8.5	7.34 10.5
Ceratopogonoid	2.76 7.7	0.84 3.5
Oligochaeta	0.30 0.8	1.13 1.4
Caddis larvae	1.73 0.8	0.79 2.1
Insecta nymph	0.18 1.5	3.32 4.9
Subimagines	0.43 1.5	1.82 4.2
Asellus aquaticus	0.30 0.8	0 0
Gastropods	0 0	1.58 1.4
Fish eggs	1.84 3.9	2.77 3.5
Algae, Plant-Tissues and Sand	0.40 3.1	0.60 5.6
Unidentified	1.3 1.5	2.77 2.1

Table 6.4: Electivity indices of the most common prey of zooplankton and benthos eaten by Loch Lomond and Balmaha Pond fish in the natural habitats. + = positive selection, - = negative selection, 0 = random selection.

FOOD TYPE	LOCH LOMOND	BALMAHA POND
ZOOPLANKTON:		
Copepods	+	+
Chydorids		+
BENTHOS:		
Chironomid larvae	-	-
Chironomid pupae	+	+
Ceratopogonoid larvae	-	
Oligochaeta		-
Caddis larvae	-	
Insecta nymphs		-
Subimagines		0
Gastropoda		-

6.3.4 Discussion

The results obtained in this section show clear differences in the food available in Loch Lomond and Balmaha Pond. Loch Lomond has a higher density of zooplankton than Balmaha Pond which in turn has a higher density of benthos. This difference in the availability of food coincided with difference in the feeding habits of sticklebacks from these two sites.

Sticklebacks from Loch Lomond feed predominantly on zooplankton whereas those from Balmaha Pond feed predominantly on benthos. However, Balmaha Pond fish still take some zooplankton (about 12% of the bulk in total, Table 6.3) despite its scarcity in the pond. The diets of Loch Lomond and Balmaha Pond fish are therefore in agreement with those described for limnetic and benthic sticklebacks respectively (eg. Lavin and MacPhail 1986), with limnetic fish feeding on zooplankton and benthic fish feeding on benthos.

However, both Loch Lomond and Balmaha Pond fish show a positive selection for zooplankton in their natural habitats. So, the high consumption of benthos by Balmaha Pond fish in their natural habitat indicates that such fish are forced to eat benthos because of the low availability of zooplankton. This preference for zooplankton can be explained by the fact that zooplankton prey have a number of visual properties, such as colour and movement, that enhance their attractiveness to sticklebacks (Chapters 4 and 5). In addition, feeding on zooplankton gives higher energetic return than feeding on benthos (Chapter 3).

6.4 HABITAT USE AND DIET COMPOSITION OF LOCH LOMOND AND BALMAHA POND FISH FEEDING INSIDE AN ENCLOSURE IN LOCH LOMOND.

6.4.1 Material and Methods

The fish

The fish used in this test were caught in Loch Lomond and Balmaha Pond during June 1986 and kept in the laboratory for c. 4 weeks during which daily meals of Tubifex worms were provided. The fish had standard lengths of 37-40 mm and 37-39 mm for Loch Lomond and Balmaha Pond fish respectively. One day before the test, the fish were marked by red plastic rings on two of their dorsal spines to increase their visibility to the observer. These marks did not seem to affect the behaviour of the fish.

The habitats

Two adjacent habitats of Camas An Losgainn Bay (Loch Lomond) were used in this study - a vegetated and a sandy (both described in chapter 5; Page 111). These two habitats were chosen because they differ both in food availability (Chapter 5) and available vegetation cover. The water (25-30 cm deep) was clear and quiet enough for the observer to witness the movement of the fish.

The enclosure

A trapeziodal enclosure (0.3x3x2.5 m) made of green netlon mesh (6 mesh/cm^2) was used to surround the two habitats. Its diagonal matched the boundary between the habitats, so it defined two symmetrical areas (2.06 m^2 each) of vegetation and sand. This shape of enclosure was

chosen to allow an observer behind the small base to see the fish in any part inside the enclosure.

Test protocol

At the beginning of the test, the enclosure was fixed in place and a single stickleback (deprived of food for 24 h) was gently released inside the enclosure on the boundary of the two habitats. The movement of the stickleback was observed for 20 min after it began skulling using the pectoral fins (typical of undisturbed fish). The times spent in the vegetated habitat as opposed to the sandy habitat and on the lake-bed (within 5 cm above the substrate) as opposed to the water column were recorded (to the nearest 1 sec) on a tape recorder. At the end of each test the stickleback was caught by a hand net, killed immediately and preserved for later stomach analysis. The test was then run on the next fish. Overall, 17 fish from Loch Lomond and 10 fish from Balmaha Pond were tested within 3 days. Two of Loch Lomond fish were lost from view frequently when under test and were therefore used only in stomach analysis.

Food availability and food consumption in the enclosure

A week after testing Loch Lomond and Balmaha Pond fish, a 20 l water sample and two core samples were taken (see above) to represent the zooplankton and benthos in vegetated and sandy habitats (the period of a week was left before sampling the food to allow testing another group of fish for the effect of predation risk on behaviour, see Chapter 7). The potential prey were identified to the lowest possible taxon. The samples were collected from the enclosure at the

end of the test and no attempt was made to take samples during the test since this would disturb the substrate structure. It was assumed that feeding did not cause a significant depletion in the amount of food available because the amount of food eaten by the fish was extremely small compared to that available in the habitats.

The stomach contents of the tested fish were identified as either zooplankton or benthos (see above) and the fish were then classified as having fed on zooplankton, benthos or zooplankton plus benthos.

6.4.2 Data analysis

A Mann-Whitney U-test was used to test the differences between Loch Lomond and Balmaha Pond fish in the time spent in vegetation v sand and in water column v lake bed. A X^2-test was used to test the differences between vegetated and sandy habitats in the number of zooplankton and benthos sampled, and the difference between the number of fish from Loch Lomond and Balmaha Pond that fed on zooplankton, benthos or zooplankton plus benthos. In the latter case of using X^2-test, some of the expected numbers in the data were less than 5. Expected numbers of less than 5 tend to increase X^2 value and increase the chance of a significant result. Since the outcome of this test was not significant (see below) these values have not invalidated the test.

6.4.3 Results

Time spent in various divisions of the enclosure

As Fig. 6.1a shows, Loch Lomond fish spent more time in the vegetated habitat than Balmaha Pond fish which in turn spent more time in the sandy habitat (these differences were not significant). Regarding the use of water column v lake bed (Fig. 6.1 b), Loch Lomond fish significantly spent more time in the water column than Balmaha Pond fish (which spent more time on the lake bed).

Food available and food eaten in the enclosure

Table 6.5 shows that the vegetated habitat was richer with prey types and had much higher density of benthos than sandy one (see also Chapter 5). However, the number of zooplanktonic prey types and their densities were similar in the water body above these two habitats.

Table 6.6 shows that the number of fish that fed on zooplankton, benthos or zooplankton plus benthos did not differ significantly between Loch Lomond and Balmaha Pond fish, both fish groups fed predominantly on zooplankton.

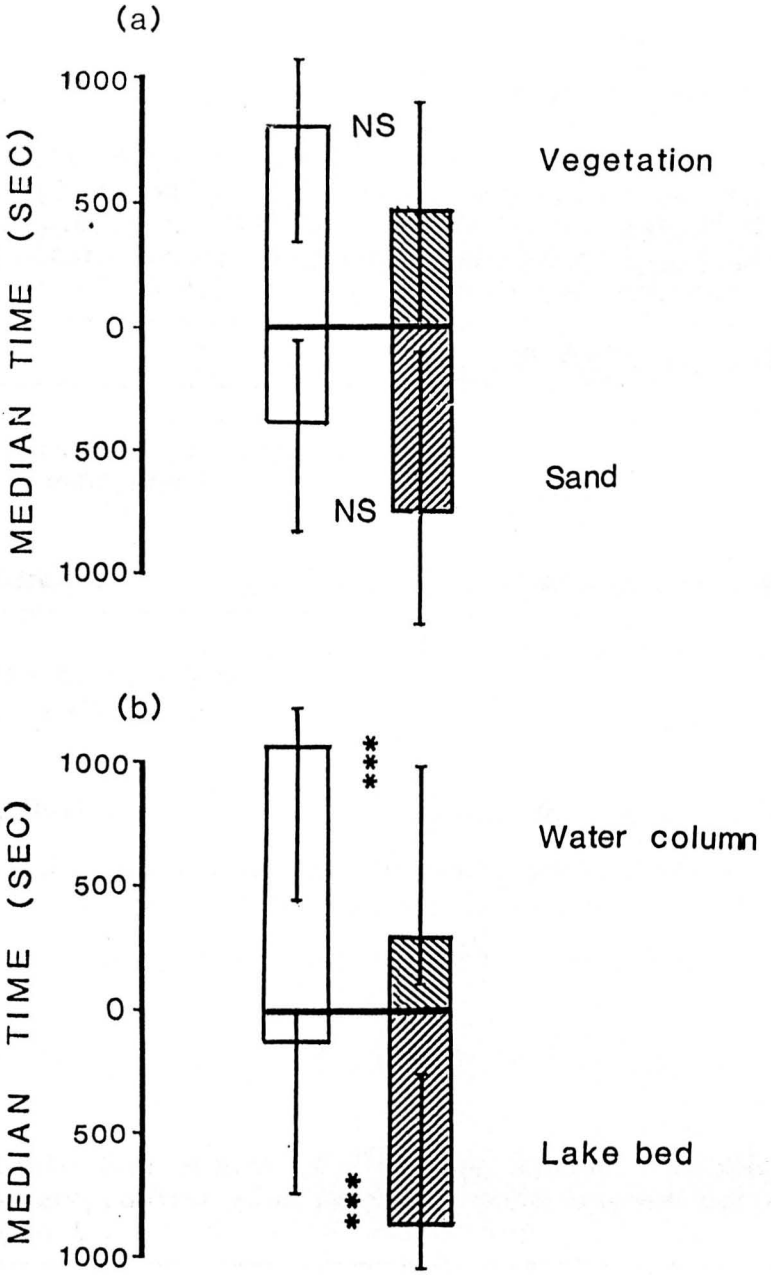

Fig. 6.1: Median and range of time (sec) spent by LochLomond (blank column) and Balmaha Pond fish (lined column) in vegetation v sand (a) and in water column v lake bed (b). *** = P <0.001, NS = not significant; U-test.

Table 6.5: Number of prey types and density of zooplankton (No. per 20 l water) and benthos (No. per sample; i.e. 0.141 m^2) sampled from vegetated and sandy habitats inside the feeding enclosure in Loch Lomond. (*** = P <0.001, NS = not significant, X^2-test)

	VEGETATED	SANDY	
NUMBER OF ZOOPLANKTONIC PREY TYPES	6	5	
DENSITY	80	60	NS
NUMBER OF BENTHIC PREY TYPES	12	5	
DENSITY	61	22	***

Table 6.6: Number of fish that fed on zooplankton, benthos or zooplankton plus benthos inside the feeding enclosure in Loch Lomond. (NS = not significant, X^2-test)

FOOD TYPES	LOCH LOMOND (n = 17)	BALMAHA POND (n = 10)	
ZOOPLANKTON	15	5	
BENTHOS	0	3	NS
BENTHOS+ZOOPLANKTON	2	2	

6.4.4 Discussion

The results of this test show that Loch Lomond fish use the water column more than Balmaha Pond fish, which in turn use the lake bed. The relatively greater use of the lake bed by Balmaha Pond fish may reflect their behaviour in their natural habitat where they inhabit a densely-vegetated habitat and feed on benthos (which mostly inhabit the lake bed). Regarding the use of vegetation and sandy habitats, Loch Lomond fish spend more time in vegetation than Balmaha Pond fish (this difference is not significant). The greater tendency of favouring the vegetated habitat by Loch Lomond fish may reflect their tendency to select the area which provides both sufficient food and shelter from predators (Chapter 7). Balmaha Pond fish are less exposed to predation risk than Loch Lomond fish (see below), and this may explain their greater tendency to use sandy, unsheltered habitat.

Both Loch Lomond and Balmaha Pond fish feed on zooplankton inside the enclosure where the density of such food (and the density of benthos) is considerably high. In contrast, in their natural habitat, Balmaha Pond fish feed predominantly on benthos (see above), this indicates that they are forced to eat benthos since zooplankton is rare in Balmaha Pond.

6.5 MORPHOLOGY OF FEEDING AND DEFENCE APPARATUSES

6.5.1 Material and Methods

Information about the morphology of each of Loch Lomond and Balmaha Pond fish was obtained by examining 25 fish chosen at random from the same fish used in feeding-habit studies (see above). Standard length (distance from the tip of the snout to the base of the caudal peduncle) and gape size (the distance between the two side-angles of the mouth) were measured. Gillraker number, length and spacing were measured on the anterior section of the first gill arch of the left side of the head. Gillrakers were counted and then the longest three were measured as the distance from the ventral margin of the base to the tip, and the mean was obtained. The widest three spaces between the gillrakers (adjacent to the longer gillrakers) were measured, as the space confined between the base of a given gillraker and the one next to it, then the mean was obtained.

The number of lateral bony plates on both sides of the body was counted. Some partially and fully plated sticklebacks were caught among Loch Lomond fish but were not included in the calculations. The longest dorsal spine (the middle spine) and the left ventral spine were also measured as the distance between the base and the tip; some of the fish had broken spines, so such spines were noted but only intact ones were measured. All the above measurements were taken to the nearest 0.05 mm, except that of standard length which was taken to the nearest 0.5 mm.

Gillraker and spine measurements as well as gape size were found to correlate with the standard length of the fish. This raises the

possibility that any difference between populations might be an indirect result of any differences in overall body size rather than a direct result of ecological condition. Therefore, in addition to the absolute values, relative values expressed as proportions of the standard lengths were calculated. Data of gillrakers counts were not standardized in this way since they stabilize in fish longer (S.L.) than 3 cm (Penczak 1965), and all fish tested were longer than 3 cm.

6.5.2 Data analysis

A Mann-Whitney U-test was used to test the differences between Loch Lomond and Balmaha Pond fish in various morphological parameters under investigation.

6.5.3 Results

The data of various morphological features of Loch Lomond and Balmaha Pond fish are presented in Table 6.7. Loch Lomond fish were larger in size and had narrower gapes than Balmaha Pond fish. Gillraker number and length were higher in fish from Loch Lomond than in fish from Balmaha Pond but the distance between the gillrakers was wider in Balmaha Pond fish, both for absolute and relative measurements.

Both dorsal and ventral spines were longer (both by absolute and by relative values) in Loch Lomond fish compared to Balmaha Pond fish, but lateral plate number (on both sides of the body) did not differ significantly between fish from the two sites. Both Loch Lomond and Balmaha Pond fish had median lateral plate of 5 on both sides of the body. Loch Lomond fish seem to have experienced direct contact with predators; 3 fish out of the 40 from Loch Lomond (7.5%) had broken dorsal spines and one (2.5%) had a broken ventral spine. In contrast, Balmaha Pond fish examined had no broken spines.

Table 6.7: Median absolute and relative values of various morphological features of Loch Lomond and Balmaha Pond fish. The range is given in parentheses. (all the measurements are in mm)
(**=P<0.01, ***=P<0.001, NS= not significant, U-test)

PARAMETERS	LOCH LOMOND FISH	BALMAHA POND FISH	
a-Fish size:			
STANDARD LENGTH	40.5 (36-44)	36.0 (33-39)	***
b-Feeding apparatus:			
ABSOLUTE GAPE SIZE	2.700 (2.4-3.1)	3.400 (3.0-3.9)	***
RELATIVE GAPE SIZE	0.066 (0.06-0.07)	0.091 (0.08-0.11)	***
GILLRAKERS NUMBER	19.00 (17-21)	16.00 (15-19)	***
ABSOLUTE GILLRAKER LENGTH	0.816 (0.66-1.0)	0.600 (0.53-0.8)	***
RELATIVE GILLRAKER LENGTH	0.020 (0.017-0.024)	0.016 (0.011-0.019)	***
ABSOLUTE GILLRAKER SPACING	0.174 (0.15-0.2)	0.200 (0.18-0.25)	**
RELATIVE GILLRAKER SPACING	0.004 (0.003-0.005)	0.005 (0.004-0.006)	***
c-Defensive apparatus			
ABSOLUTE DORSAL SPINE LENGTH	4.000 (3.1-4.5)	3.000 (2.2-3.3)	***
RELATIVE DORSAL SPINE LENGTH	0.093 (0.08-0.1)	0.079 (0.06-0.09)	**
ABSOLUTE VENTRAL SPINE LENGTH	5.450 (4.3-6.0)	4.400 (3.8-4.9)	***
RELATIVE VENTRAL SPINE LENGTH	0.129 (0.11-0.15)	0.122 (0.10-0.14)	**
LEFT LATERAL PLATE NUMBER	5.000 (4-7)	5.000 (4-6)	NS
RIGHT LATERAL PLATE NUMBER	5.000 (4-7)	5.000 (4-7)	NS

6.5.4 Discussion

The results show that Loch Lomond and Balmaha Pond fish differ in the morphology of their feeding and defence apparatuses. These differences can be related to differences in food available and/or in predation risk, but since the two populations differ in both these respects and in many other ecological properties (see above), only tentative conclusion can be drawn about the additional consequences of the morphology and behavioural differences. Loch Lomond fish have a larger body size, smaller gape, and more numerous, longer and closer gillrakers than Balmaha Pond fish. The larger body size of Loch Lomond fish may be a result of the more profitable food they eat (i.e. zooplankton, Chapter 3) or may reflect the adaptation of fish to predation pressure, since larger body size may function as a defensive mechanism against predators (Moodie 1972). The wider gape of Balmaha Pond fish may enable them to exploit benthos (the major food type in their natural habitat) more efficiently than Loch Lomond fish. The narrower gape and more numerous, longer and closer gillrakers of Loch Lomond fish may enable them to take zooplankton more efficiently (Zaret 1980, see also Chapter 3). In respect of association between feeding apparatus morphology and diet (see above), Loch Lomond and Balmaha Pond sticklebacks resemble the previously described 'limnetic' and 'benthic' sticklebacks respectively (Lavin and MacPhail 1986).

Loch Lomond sticklebacks have longer dorsal and ventral spines than Balmaha Pond fish. Larger spines have been frequently reported to be associated with higher predation level upon stickleback populations (Gross 1978, Giles 1987 b). Moreover, some of Loch Lomond fish have broken spines (either dorsal or ventral) whereas Balmaha Pond fish

(which have no contact with predatory fish) have no broken spines. The broken spines of fish caught from Loch Lomond may well be the result of unsuccessfull attacks by predators.

The lateral plate number does not differ significantly between Loch Lomond and Balmaha Pond fish, both fish having a median lateral plates number of 5 per side. The present result therefore does not agree with the association between lateral plates number of 7 and estimated level of predation risk found in sticklebacks of North-American origin (Hagen and Gilbertson 1972, 1973). However, it confirms earlier work on sticklebacks from European origin (eg. Giles 1987 b). Thus, the observation of the morphology of the defensive apparatus of fish from Loch Lomond and Balmaha Pond agrees well with the estimated level of predation risk in these sites, and both lead to the conclusion that Loch Lomond fish undergo a high predation pressure and Balmaha Pond fish undergo a low predation pressure.

6.6 CONCLUSIONS

1. Zooplankton is available in Loch Lomond more than in Balmaha Pond, whereas benthos is available in Balmaha Pond more than in Loch Lomond.

2. In their natural habitats, Loch Lomond fish feed predominantly on zooplankton and Balmaha Pond fish feed predominantly on benthos. However, the results show that both fish groups prefer zooplankton to benthos (as indicated by the electivity indices).

3. Loch Lomond fish prefer water column habitat to lake bed habitat whereas Balmaha Pond fish do the reverse. However both fish groups feed predominantly on zooplankton when offered a choice in an enclosure in Loch Lomond.

4. Although only two sites, differing in many ecological properties, have been used in this comparison, giving existing knowledge about variation in behaviour and morphology in stickleback populations, some tentative conclusions can be drawn. Thus, comparison of the behaviour and the morphology of Loch Lomond and Balmaha Pond fish together with the existing literature suggest that Loch Lomond fish resemble the previously described 'limnetic' sticklebacks and Balmaha Pond fish resemble the 'benthic' sticklebacks. Loch Lomond fish undergo a high predation pressure whereas Balmaha Pond fish undergo a low predation pressure.

CHAPTER 7

EFFECT OF PREDATION RISK ON FEEDING BEHAVIOUR OF STICKLEBACKS

7.1 INTRODUCTION

7.1.1 Feeding behaviour and predation risk

There is an increasing interest in the selective forces that mould the behaviour of the foraging animals as they deal with the task of finding and consuming their food. Animals require food in order to maintain growth and reproduction and their fitness will be critically influenced by how efficiently they obtain this. Classical Optimal Foraging Theory (MacArthur and Pianka 1966, Emlen 1968, Estabrook and Dunham 1976, Krebs et al. 1983, Krebs and Davis 1987) predicts that animals should adopt those foraging strategies that provide the highest energy rewards per unit cost, which is usually measured in terms of time. Many empirical tests have shown this to be the case (eg. Werner and Hall 1974, Gibson 1980).

However, time may not be the only important cost that animals seek to minimize; the need to avoid predators is also important. In the short term, foraging animals of various species sacrifice food intake in order to avoid predators (Milinski 1986 b). Thus, feeding behaviour of an animal may be affected by the presence of predators and this effect can come about in various ways:

a. Effect of predators on time of feeding:

Foragers may avoid feeding at dangerous times; many diurnal fish seek shelter and stop searching for food at dusk when their vulnerability to predators is high, only recommencing feeding later in the morning (Helfman 1981, 1986).

b. Effect of predators on choice of feeding site:

Foragers may avoid profitable but dangerous food patches, sacrificing food rewards in order to avoid predators. This has been shown by many studies (Fraser and Cerri 1982, Werner et al. 1983, Power 1984, Fraser and Huntingford 1986). Cerri and Fraser (1983) tested minnows (Rhinichthys atratulus) in two artificial streams each divided into several compartments, some of which contained adult minnows, Semotilus atromaculatus, as a predator. All the compartments in the first stream had a low level of food whereas predator compartments in the second stream contained additional food. During the 6 days of the test, minnows were frequently found only in the predator compartments which had high food level. This indicated that the fish avoided poor but risky sites, but the high food availability induced some fish to take risk.

Relatively few studies on the effects of predators on foraging have been conducted in the field. Power (1984) found that species of catfish (Loricariidae) occurred in deeper water than would be expected from distribution of their main prey. The conclusion was that catfish migrated to deeper water in response to the activities of their predator, a wading bird (Butorides striatus virescens) which feeds mostly in shallow water.

c. Effect of predators on foraging once in a feeding site:

Animals may forage less efficiently when in a good patch, usually because of the need for vigilance (Milinski and Heller 1978, Milinski 1984, Dill and Fraser 1984, Lawrence 1985, Lima and Valone 1986). Miliniski (1984) suggested that the ability of the nervous system of animals to process two different types of sensory information will be reduced as each of the two tasks

becomes more difficult. He showed that three-spined sticklebacks feeding on high-density swarms of Daphnia sp. were more likely to overlook an approaching predator than those feeding on low density swarms. Dill and Fraser (1984) investigated the influence of a predator (model rainbow trout) on the distance at which juvenile coho salmon (Oncorhynchus kisutch) would swim upstream from holding stations to take drifting prey items of various sizes. They found that salmon foraging in the presence of a potential predator reduced the attack distance and consequently reduced the time spent moving, particulary in response to the largest prey items. Moreover, higher frequencies of presentation of the predator resulted in further reduction in the attack distance. So, juvenile coho salmon probably captured fewer and smaller prey items as predation risk increased and thus suffered reduction in energy intake.

Lawrence (1985) showed that blackbirds (Turdus merula L.) searching for cryptic prey are less likely to detect a predator than blackbirds searching for conspicuous prey. Thus, the difficulty in discriminating between prey items may impose pressure on feeding behaviour of animals when under predation threat. Metcalfe (1984) suggested that feeding on prey types with short handling times allows purple sandpipers (Calidris maritima) to scan for predators more efficiently than feeding on prey types with longer handling times. Thus, animals feeding on easy-to-handle prey are potentially better at avoiding attacks by their predators. As an illustration of the converse effect, Metcalfe et al. showed that increasing predation risk increased the reaction time of juvenile salmon (Salmo salar) to food particles (1987 a) and caused them to cease discriminating between suitable and unsuitable food items (1987 b).

The level of predation pressure on fish populations can have an effect on subsequent feeding behaviour. In sticklebacks, different populations differ in their immediate responses to predators, and such differences can be related to local predation levels (Giles and Huntingford 1984, Huntingford and Giles 1987). It is suggested, therefore, that any effect of a predator on subsequent foraging might also differ in fish from population exposed to different predation levels.

So, feeding on dense or cryptic prey appears to impair the ability of foragers to detect predators and the increased need for vigilance can decrease the ability of foragers to make usual discrimination between prey types. In addition, the local predation pressure may effect feeding behaviour.

7.1.2 Aims

This chapter deals with laboratory and field studies aimed at investigating the effects of predators on feeding behaviour of three-spined sticklebacks from two sites with different levels of exposure to predators. The specific aims are:

1. To investigate, in a laboratory test, the effect of a predator on various aspects of foraging behaviour, i.e. time taken to initiate a foraging bout, number of feeding attempts and choice between two types of prey of different profitabilities.

2. To investigate, in a field test, the effects of a predator on diet choice, amount of food eaten, and size of prey selected in sticklebacks: fish from two different populations, one is exposed to a high predation risk and another is exposed to a low predation risk, were used in order to investigate the effect of level of local predation pressure on feeding behaviour.

3. Also in the field, to study the effect of a predator on microhabitat choice and on the type of food eaten (zooplankton or benthos) using fish from Loch Lomond only.

7.2 LABORATORY STUDY ON THE EFFECTS OF PREDATION RISK ON FOOD CHOICE

7.2.1 Material and Methods

The fish

Sticklebacks were collected from the River Kelvin in March 1985 and chosen with standard lengths of 40-42 mm, because in a previous study (Chapter 4) fish of this size showed a stronger preference in favour of the most profitable prey types than smaller fish. The fish were kept at 13-16^{o}C and under 7:17 h dark-light cycle (to prevent them coming into reproductive condition) for at least three weeks before being tested. The subjects were fed daily on chopped earthworms, so the fish had no experience with the experimental prey after they were caught.

The test tank

Tests were carried out in tanks similar to those used in the profitability choice tests (described in chapter 4; Page 76).

The prey

The fish were tested on two sets of live prey, medium sized Chironomid larvae v small Asellus, and medium sized Chironomid larvae v large Chironomid larvae (see Table 3.1 for the actual sizes of these prey). These two pairs have similar profitability values (7.25 v 5.15 and 7.25 v 5.10 joules/sec respectively, Table 3.8), so the medium Chironomid larvae were always the more profitable prey items. The prey in the former set were thought to be more easily discriminated from each other than those in the latter set. Each prey was attached to the end of a capillary glass tube (1 mm in diameter) by a fine thread and suspended inside one of the test tubes. The capillary tubes were then held in place by rubber stoppers in such a way that the prey were at 1 cm height above the bottom of the test tubes.

The predator

The predator used in this test was a black silhouette of a European kingfisher (Alcedo atthis), 16 cm beak to tail, fixed on a rotating motor-arm which moved at a speed of 90 cm/sec. This model was used because the kingfisher is well known to be a predator of sticklebacks and is available in the area where the test fish were caught (Gibson 1981). In addition, such a model is known from the literature to elicit avoidance and to influence foraging behaviour in sticklebacks (Milinski and Heller 1978).

The test protocol

The fish were tested in similar manner to those in the profitability choice test (described in chapter 4; Pages 77-78). Two groups of fish were tested, one without predator (control fish) and the other with predator (experimental fish). In the experimental tests, the predator was allowed to glide 3 times above the start compartment at 38 cm just before the door was opened to give the fish access to the test compartment. In the control tests the door was opened without the fish being exposed to the predator. The time from opening the door until the stickleback entered the test compartment and then the time before the first bite at the tubes were recorded (to the nearest 1 sec) using stop watches. A record was then taken of the tube which was bitten first and, as explained in chapter 4 (Page 78), the prey contained in that tube was regarded as the preferred prey. The total number of bites made by the stickleback during the first 2 min following the first bite was also recorded. Tests with and without predator were alternated and the position of the tubes was switched regularly in order to minimize any effects of side bias. Each fish was tested only once. Overall, 84 (42 controls and 42 experimental) and 39 fish (19 control and 20 experimental) were tested on easy and difficult discrimination sets respectively.

7.2.2 Data analysis

A Mann-Whitney U-test was used to test the differences between the control and the experimental fish in the time spent until they entered the test compartment, the time delay until the first bite and the

total number of bites made to the prey. A X^2-test was used to test the differences between the number of first choices to the more profitable and less profitable prey made by the control and by the experimental fish.

7.2.3 Results

The experimental fish showed visible fright responses on seeing the model predator, raising their dorsal and ventral spines and jumping away. In addition to these responses, the experimental fish, whether facing an easy or a difficult discrimination, spent significantly longer time before entering the test chamber than the control fish (Fig. 7.1 a). Once in the test chamber, the experimental fish also hesitated significantly longer than the control fish before initiating their attacks to the tubes containing prey (Fig. 7.1 b). However, prior exposure to the predator did not alter the total number of attacks over the whole two minutes (Fig. 7.1 c).

Regarding diet choice (Fig. 7.1 d), when the discrimination between the two prey items was difficult (i.e. medium Chironomid larvae v large Chironomid larvae), the fish showed no selection in favour of the more profitable prey either in the presence or in the absence of the model predator. In contrast, when the discrimination was easy (medium Chironomid larvae v small Asellus), control fish showed a significant preference for the more profitable prey (medium Chironomid). When the model predator was present, this preference for the more profitable prey was eliminated and the distribution of the

first bites made by the fish to the two prey types was no longer significant. The differences in the first choices between the experimental and the control fish were not expected to be significant since no reversal in the choice had occurred.

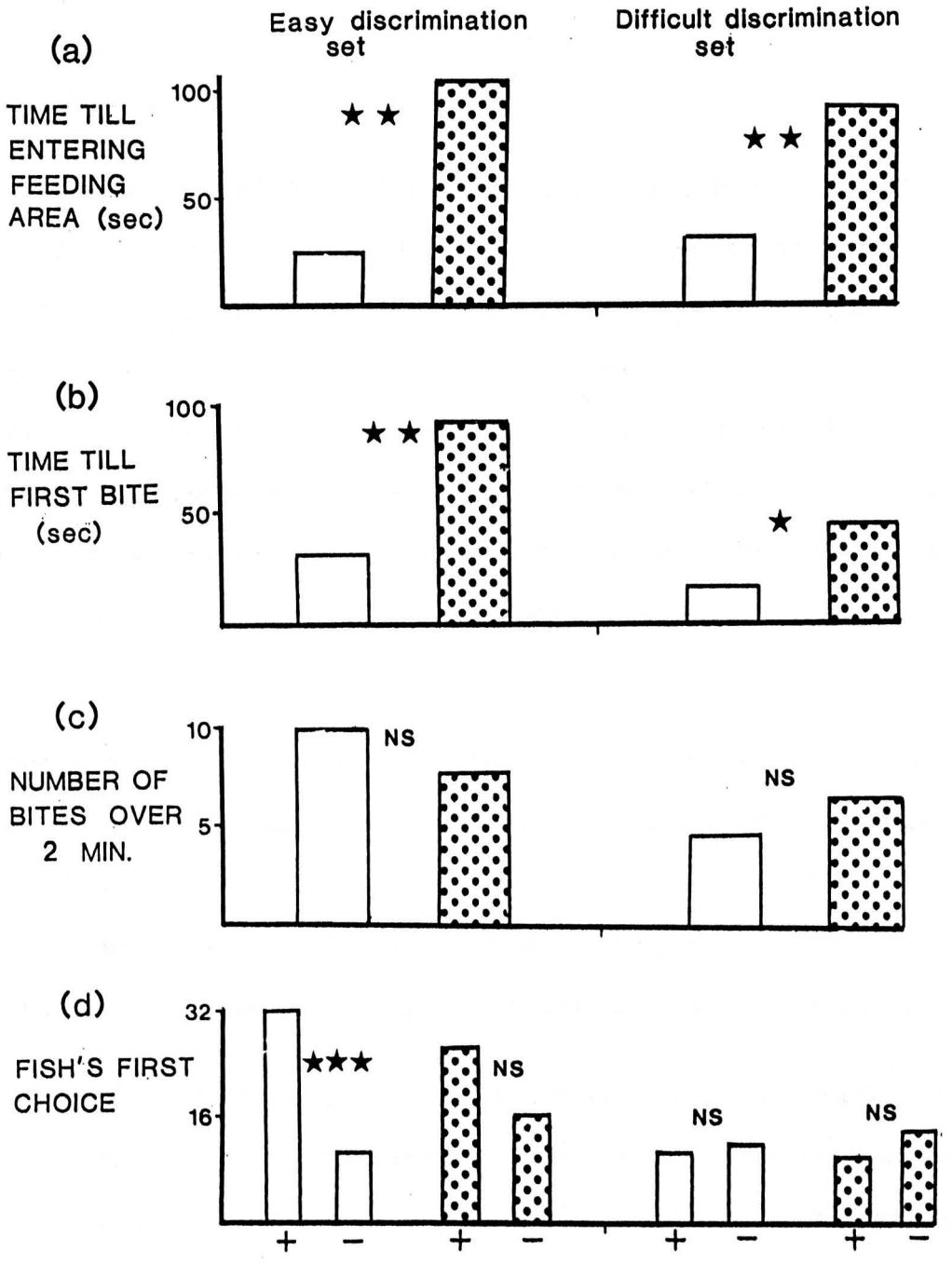

Fig. 7.1: Median time (sec) spent by control (blank column) and experimental fish (dotted column) before entering the feeding area (a) & till biting at the prey (b), median number of bites (c) and the fish's first choice to the more profitable (+) and less profitable prey (-).
(* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, NS = not significant, U-test (a, b, c) and X^2-test (d).

7.2.4 Discussion

As the previous studies (Milinski 1984, Metcalfe et al. 1987 a, b) have shown, exposure to predation risk influences foraging behaviour and does so in a number of different ways. The sticklebacks take longer to enter the foraging site and hesitate longer before biting at the prey items once in the site. In this case, the fish is giving priority to predator-avoidance rather than to feeding. An individual fish would lose everything if caught and eaten and, in the short term, should be willing to sacrifice energy intake in order to identify and avoid the predator.

Since the overall number of bites is not reduced by exposure to predator, fish foraging at a predation risk must be compensating for their slow start by higher subsequent rate of bites once feeding begins. However, energy intake will still be reduced since discrimination in favour of the more profitable prey is impaired.

The effects of predation risk on the behaviour of sticklebacks biting at the difficult discrimination set of prey (medium Chironomid larvae v large Chironomid larvae) are similar to those for the easy discrimination set (medium Chironomid v small Asellus), except that fish do not seem to be able to discriminate between the two prey either in the presence or in the absence of the predator. This could be attributed to the similarity of the body characteristics (such as colour, movement and shape) of the two prey. These prey differ in size but this cue is known to be of the least importance in the food choice in sticklebacks (Chapter 4). Where sufficiently clear cues for discrimination are available, and in the absence of a predator, the fish become able to assess the value of the food and to choose

the more profitable prey item (see also Chapter 4). However, in the presence of the predator, the discrimination between the more and the less profitable prey disappears. As the results in chapter 3 shown, small Asellus have both a longer handling time (3 sec) and a smaller energetic value (15.48 joules) than medium Chironomid (2.55 sec, 18.49 joules respectively). Thus, by failing to discriminate against Asellus, the experimental fish have lost out in terms of both time and energy. So, faced with the incompatible need of vigilance and prey selection, sticklebacks give priority to vigilance at the cost of efficiency in feeding.

7.3 FIELD EXPERIMENT ON THE EFFECT OF PREDATION RISK ON FOOD CHOICE IN LOCH LOMOND AND BALMAHA POND STICKLEBACKS

7.3.1 Material and Methods

The fish

The sticklebacks used in this test were caught in Loch Lomond and Balmaha Pond during July 1986, housed in the laboratory for 4 weeks prior to the test and fed daily on Tubifex worms. These two fish groups had standard lengths of 37-40 mm and 36-39 mm respectively.

The environment

Tests were run on a 60 m^2 area of vegetated substrate located in Camas An Losgainn Bay, Loch Lomond. The characteristics of this

substrate were described in chapter 5 (Page 111). The water above this substrate (45 ± 5 cm) was clear, moderately quiet, well oxygenated (95-100 % saturated), and had a temperature of 13-16°C (the last two features were measurred by Mackereth combined Thermo-Oxygen meter).

The feeding enclosures

The tests were run in two identical enclosures, one was used for the test without a predator (control) and the other for the test with a predator (experimental). Each enclosure measured 1.25x1.25x0.65 m and was made of green netlon mesh (6 mesh/cm^2). The enclosures were supported by metal rods at the corners to keep the desired shape.

The predator

The predator used in this test was a 20 cm (S.L.) live rainbow trout (Salmo gairdneri) brought from a domestic farm, kept for 3 weeks under running loch water and fed twice a day on small live sticklebacks and minnows. During the test the trout was placed inside the feeding enclosure enclosed in a transparent perspex tank (45x25x30 cm) which had its two narrower sides and its top replaced with white mesh (3 mesh/cm^2). This tank was positioned in such a way to allow a free movement of the water through it. The water current then caused the trout to keep moving and thus presented a more constant stimulus. This design of the tank, which increased the activity of the predator, and the 24 h intervals between tests (see below) were thought to be necessary because previous experiments, in which the trout was enclosed in a normal tank (with no mesh walls were provided) and with no considerable intervals between tests, did not show any effect due to the presence of the predator on the feeding parameters under

consideration (see below). The tank was supported 10 cm above the substrate with a transparent perspex frame, so that the trout could be seen by sticklebacks from any point within the enclosure.

The test protocol

One day before each test the enclosures were arranged on the substrate, the tank containing the trout (which had already been fed) was centered into one of the two enclosures and left until the following day. Three individuals of sticklebacks (deprived of food for 24 h) were then gently released into each enclosure (taking care not to disturb the predator) and left to feed for 20 min on the array of zooplankton and benthos available. This feeding period was long enough for the fish to have stomachs with moderate fullness (20-40% of the full capacity, pilot studies) but short enough to minimize prey depletion. After 20 min, the test was terminated and the fish were caught by hand net, killed immediately and preserved for the subsequent analysis. The enclosures were then moved to new positions in preparation for the next test which was carried out on the following day. Thus, about 24 h were left between tests to allow resettling the trout. The locations of the enclosures were distributed all over the substrate with the requirement that no one position was used twice. Overall, 54 fish from Loch Lomond and 30 fish from Balmaha Pond were tested over a period of 14 days where half of the fish were tested with and half without the predator. Tests with and without predator were run simultaneously to reduce any effects of fluctuation in the environmental conditions.

Stomach contents analysis

The stomach contents of the tested fish were quantified according to bulk method described in chapter 5 (Pages 130-131). The number of fish whose stomachs contained at least one item of a given food type was recorded. The longest dimension (excluding appendages) of each food item was measured (to the nearest 0.05 mm) to indicate its size. When stomachs contained a large number of items from the same taxon, a random sample of 20-40 items was measured to indicate the size distribution of that taxon.

7.3.2 Data analysis

Amount of food eaten

The bulks of the food types in each stomach were summed to give the total amount of food eaten (i.e. stomach fullness; measured in terms of weight units, see chapter 5; Page 130). A Mann-Whitney U-test was then used to test the difference in the amount of food eaten between the experimental and the control fish.

Choice of prey types

The percentage by bulk of the most common food type (Bosmina coregoni) in each stomach was calculated and a Mann-Whitney U-test was used to test the difference between the amounts eaten by the control and experimental fish. Other prey types were not considered in this way because they provided no enough data to employ a statistical test.

A X^2-test was used to test the difference between the number of the experimental and the control fish that fed on a given food type.

Choice of prey size

Prey size measurements were categorized into the overall size of prey eaten and the size of the most abundant prey (Bosmina coregoni). Mean prey size was calculated for each stomach and a Mann-Whitney U-test used to test the differences between the experimental and the control fish in this parameter.

7.3.3 Results

Amount of food eaten

The amount of food consumed by the experimental fish was significantly lower than that consumed by the control fish both in Loch Lomond and in Balmaha Pond fish. Thus, median stomach fullness of the experimental fish was 12 and 5 units for Loch Lomond and Balmaha Pond fish respectively; this was lower than the median stomach fullness of the control fish (27 and 24.5 units respectively; $P < 0.05$ in both fish groups, U-test).

Choice of prey types

Table 7.1 shows that the control and experimental sticklebacks from Balmaha Pond ate similar food, with both groups relying to great extent on B. coregoni (i.e. the difference was not significant, U-

test). In contrast, the control and experimental sticklebacks from Loch Lomond showed some differences in their diets:

a. Fewer experimental fish ate Chironomid larvae and pupae compared to control fish (the difference was significant only in the case of Chironomid pupae; $X^2 = 7.8$, df = 1, $P < 0.01$, X^2-test) and smaller bulks of these prey were consumed by the experimental fish (these differences were not statistically tested, see above).
b. The experimental fish consumed higher amounts of Bosmina corigoni than the control fish (this difference was not significant).
c. There was an increased incidence of benthos (Plecoptera, Ephemeroptera, and Asellus aquaticus) in the diet of the experimental fish, while no control fish ate any of these prey types.

Choice of prey size

Table 7.2 presents the median sizes of prey consumed by the control and the experimental fish from Loch Lomond and Balmaha Pond. The presence of the predator did not influence the choice of prey size in Balmaha Pond fish, the diet of both control and experimental fish consisted of Bosmina. In contrast, in Loch Lomond fish, the experimental fish consumed prey of smaller sizes than the control fish. This difference emerged because:

a. The experimental fish shifted their diet away from larger prey items (Chironomid larvae and pupae, see above) and thus their diet was predominanted by smaller items (Bosmina).

b. Considering only Bosmina (the most abundant food type), the experimental fish ate smaller prey items than the control fish.

Table 7.1: Percentage by bulk (upper figure) and frequency of occurrence (lower figure) of various food types eaten by control and experimental fish from Loch Lomond and Balmaha Pond fish.

FOOD TYPE	LOCH LOMOND FISH		BALMAHA POND FISH	
	Without predator n=27	With predator n=27	Without predator n=15	With predator n=15
ZOOPLANKTON:				
Bosmina coregoni	58.40 24	83.46 22	97.03 12	94.2 9
Chydoridae	1.22 4	0.65 4	- -	0.4 2
Daphnia sp.	0.44 1	0.65 2	- -	- -
Cyclops spp.	0.08 1	1.43 1	- -	- -
BENTHOS:				
Chironomid larvae	25.11 7	4.68 3	2.04 1	1.5 1
Chironomid pupae	10.50 9	1.04 1	- -	1.1 1
Caenis sp.	4.01 1	5.2 1	- -	- -
Ephemeroptera nymph	- -	1.04 1	- -	- -
Plecoptera nymph	- -	0.39 1	- -	- -
Corixid nymph	- -	- -	0.63 2	1.6 2
Asellus aquaticus	- -	0.39 1	- -	- -
Gastropoda	- -	- -	0.36 1	1.4 1
Plant tissues	0.15 1	0.4 1	- -	- -

Table 7.2: Median (and range) overall prey size and Bosmina's size in the stomachs of control and experimental fish from Loch Lomond and Balmaha Pond fish feeding inside enclosures in Loch Lomond. (** = P <0.01, NS=not significant, U-test)

	LOCH LOMOND FISH			BALMAHA POND FISH		
	Control	Experimental		Control	Experimental	
Median overall prey size (mm)	0.65 (0.43-5.8)	0.48 (0.35-2.8)	**	0.51 (0.4-1)	0.50 (0.4-0.8)	NS
Median Bosmina's size (mm)	0.50 (0.42-0.61)	0.46 (0.34-0.55)	**	0.49 (0.4-0.55)	0.46 (0.4-0.6)	NS

7.3.4 Discussion

The results of this experiment show that increased predation risk affects the amount of food, the type of prey, and the size of prey eaten by sticklebacks foraging in the field. Both Loch Lomond and Balmaha Pond sticklebacks eat reduced amounts of food when foraging in the presence of a predator. This could be the result of a temporary suppression in feeding, of a microhabitat change (for example, the fish may forage closer to shelters) or of the need to remain vigilant (Milinski 1984, and see above). Not incompatible, the additional changes in foraging shown in response to predation risk by Loch Lomond sticklebacks indicate that the last process (increased vigilance) may well be in operation. Loch Lomond sticklebacks have altered their diet by shifting away from Chironomid larvae and pupae to Bosmina and from larger items to smaller items within this latter prey type. A

small-scale supplementary test of measuring the profitabilities (see the method in chapter 3, Pages 31-36) of these prey types has shown that Bosmina have shorter handling times (mean = 0.32 ± 0.05 sec, S.E.) than Chironomid larvae (mean = 2.7 ± 0.30 sec; P <0.001, Two-sample t-test) and Chironomid pupae (mean = 2.39 ± 0.25 sec; P <0.001, Two-sample t-test). In contrast, both Chironomid larvae and pupae are more profitable (profitabilities value = 0.80 and 0.51 joules/sec respectively) than Bosmina (profitability = 0.17 joules/sec). So the fish have shifted their diet from difficult-to-handle but more profitable prey to easy-to-handle but less profitable prey when under predation threat. Such a change in the diet is known to increase the ability of foragers to detect predators (Metcalfe 1984, Milinski 1984, Lawrence 1985). The shift away from Chironomid larvae and pupae by the experimental sticklebacks might also represent a reduced tendency to take up the head-down posture required to feed on benthos. Such a posture would make them lose sight of the predator (Milinski 1985) and consequently enhance the risk of capture. Selecting smaller sizes of Bosmina by Loch Lomond sticklebacks feeding in presence of the predator results in a reduction in the energetic intake. This reduction arises because smaller prey are less profitable than larger prey since handling times of these two sizes of prey are similar (Werner and Hall 1974, Mittelbach 1981, see also Chapter 3).

The results suggested that Balmaha Pond fish were less affected by predation risk than Loch Lomond fish. Although they ate reduced amount of food in the presence of the predator and their diet consisted of smaller prey (Bosmina), Balmaha Pond fish, unlike Loch Lomond fish, did not show a shift either in the type or in the size of the prey eaten in response to the threat. Since prey discrimination impairs vigilance (see above), the indiscrimination in favour of

larger prey by Loch Lomond fish feeding in the presence of the predator might indicate that vigilance process is still in operation. Balmaha Pond fish showed no similar reduction in size of Bosmina eaten and this might indicate that the time devoted for vigilance was not increased in similar manner to that of Loch Lomond fish feeding in the presence of the predator.

Since Loch Lomond fish are exposed to a higher predation pressure in nature (Chapter 6), then the increased ability to modify their foraging behaviour in response to predation risk may be a part of their whole spectrum of highly developed anti-predator responses (Giles and Huntingford 1984, Huntingford and Giles 1987, Tulley and Huntingford 1987). The incomparable behaviour of Balmaha Pond fish could be attributed to the fact that they are less exposed to predation risk in their natural habitat (Chapter 6) or because their diet consisted of smaller prey anyway.

7.4 FIELD EXPERIMENT ON THE EFFECT OF PREDATION RISK ON HABITAT USE IN LOCH LOMOND FISH

7.4.1 Material and Methods

This test was carried out jointly with that of habitat use by Loch Lomond and Balmaha Pond fish (Chapter 6). Thus, the data obtained from testing Loch Lomond fish in the above mentioned test were used for the comparison with the data obtained from testing another group of fish (from Loch Lomond) in presence of a predator. These two fish groups were called the control and the experimental fish respectively. The experimental fish had similar size distribution to the control fish and were tested in similar way, on the same habitats and using the same enclosure (see Pages 178-179).

The predator used was a 20 cm (S.L.) live rainbow trout (Salmo gairdneri) enclosed in a perspex tank (described in Page 205). Due to the loss of the rainbow trout, the test was continued with a 30 cm (S.L.) live brown trout (Salmo trutta); the reaction of the sticklebacks to the two trout did not differ. A wooden board was laid on the top of the tank and extended 10 cm on each side, so the predator could not see the observer and consequently would not be disturbed.

After testing the control fish, the test was continued on the experimental fish. The tank containing the trout was centered in the feeding enclosure and individual sticklebacks were tested singly at 12 h intervals (i.e. 2 fish per day) to allow the trout to settle down after eacn test. The control and the experimental tests were

carried out in this sequence in order to minimize disturbing the predator whose behaviour was readily affected by capture and removal. Overall, 13 experimental fish were tested.

Food availability and food consumption

The food available in the habitats is already described in Table 6.5 (Page 183). The food eaten by the experimental fish was analysed in similar way to that of the control fish (Page 180).

7.4.2 Data analysis

A Mann-Whitney U-test was used to test the differences between the control and the experimental fish in the time spent in various parts of the enclosure. A X^2-test was used to test the differences between the number of control and experimental fish that fed on zooplankton, benthos or zooplankton plus benthos. Even though some of the expected numbers in the data were less than 5, X^2-test was used for the same reason outlined in chapter 6 (Page 180).

7.4.3 Results

Behaviour of the fish under test

When first released into the enclosure, both the control and the experimental fish tended to freeze for periods ranging from 30 to 180 sec before starting swimming. Experimental fish showed a marked reaction to the predator; they usually approached the tank containing

the trout, swam around for few seconds with their snout contacting the perspex, then jumped away (in unpredictable direction) and froze again for a while before resuming swimming. About 50% of the experimental fish repeated this behaviour more than once throughout the test period. A similar behaviour (which is called 'investigation behaviour') was also observed in minnows (Phoxinus phoxinus) by Magurran et al. (1985).

Time spent in various divisions of the enclosure

Fig. 7.2 a shows that control and experimental fish did not differ significantly from each other in the time spent in the vegetated and in the sandy habitats. However, regarding the time spent in the water column and the lake bed (Fig. 7.2 b), control fish spent significantly more time in the water column than experimental fish, which in turn spent more time on the lake bed.

Food available and food eaten in the enclosure

As shown in Table 6.5 (Page 183), the vegetated habitat was richer with food types and had a much higher density of benthos than the sandy habitat. However, the number of food types of zooplankton and their densities were similar in the water body of the two habitats. Table 7.3 shows that the number of fish that fed on zooplankton, benthos or zooplankton plus benthos did not differ significantly between the experimental and the control fish, with both fish groups fed predominantly on zooplankton.

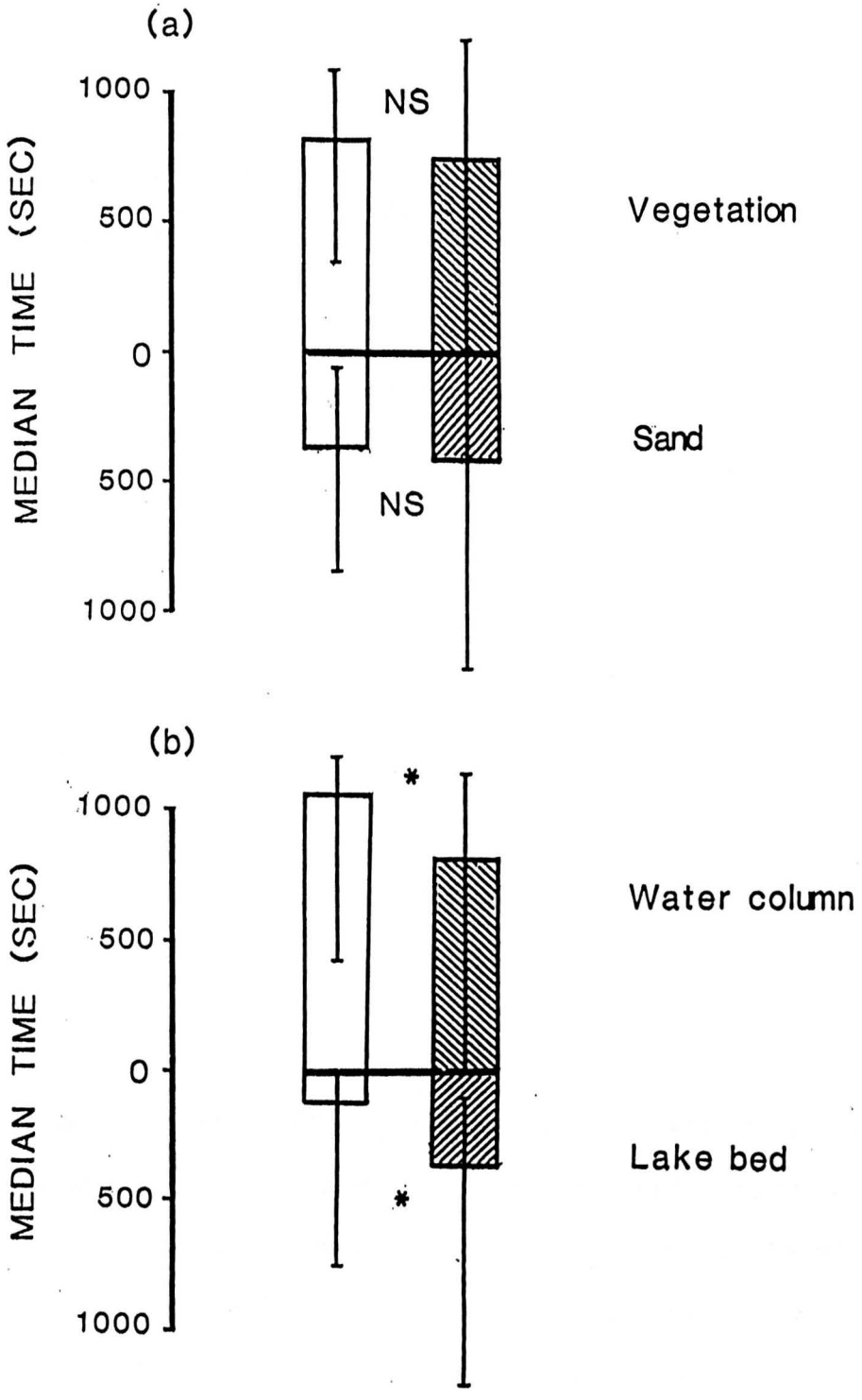

Fig. 7.2: Median (and range) time (sec) spent by control (blank column) and experimental fish (lined column) in vegetation v sand (a) and in water column v lake bed (b). * = P <0.05, NS = not significant, U-test.

Table 7.3: Number of control (rewritten from Table 6.6, for the comparison) and experimental fish that fed on zooplankton, benthos or zooplankton plus benthos inside the feeding enclosure in Loch Lomond.
(NS = not significant, X^2-test.)

FOOD TYPE	CONTROLS	EXPERIMENTALS	
ZOOPLANKTONIC	15	11	
BENTHIC	0	2	NS
BENTHIC + ZOOPLANKTON	2	0	

7.4.4 Discussion

The presence of the predator does not seem to affect the use of the vegetated and sandy habitats by sticklebacks; both experimental and control fish spending most of their time in the vegetated habitat. This habitat is found to be richer in food than the sandy habitat (at least to the extent of having more benthos) and consequently may provide a better supply of food. In addition, the vegetated habitat may provide a good shelter from predators (Savino and Stein 1982, Werner et al. 1983). However, the presence of the predator does affect the vertical distribution of the fish; the control fish prefer the water column more than the experimental fish. The water column microhabitat is known to be more valuable than the substrate, since

sticklebacks are more efficient at feeding on the organisms that live in such area (zooplankton) compared to the organisms that live on the lake bed, benthos (Chapter 3). In this case, by shifting the microhabitat away from the water column, the experimental fish probably reduce their feeding efficiency. However, by making this shift, they probably derive protection from the shelter on the lake bed.

The presence of the predator does not alter the broad choice of the fish to the food types, both experimental and control fish feeding predominantly on zooplankton. However, few experimental fish have fed exclusively on benthos, while none of the control fish has done so (Table 7.3). The fact that the experimental fish still feed on zooplankton, even though they reduce the use of the water column, indicates that they might have been feeding just above the vegetation where zooplankton are available and where it is a matter of only few centimeters to get back to the cover. Alternatively, feeding may have been suppressed when the fish were hiding on the lake bed.

7.5 CONCLUSIONS

1. In laboratory tests, predation risk slows the response of sticklebacks to food and reduces the ability to discriminate between food items of different profitabilities. The number of feeding attempts has not been affected.

2. In a field experiment on diet choice of Loch Lomond (which are exposed to high predation risk) and Balmaha Pond fish (which are exposed to low predation risk), predation risk has suppressed the amount of food eaten by both fish groups. Loch Lomond fish, unlike Balmaha Pond fish, have responded further to the predator by selecting different prey types and smaller prey sizes.

3. In the field, Loch Lomond sticklebacks increase the use of the lake bed and avoid the water column when under predation threat, although they feed predominantly on zooplankton. The use of vegetation and sandy habitats has not been affected.

4. Overall, the results suggest that sticklebacks accept reduced energetic intake in favour of predator avoidance.

CHAPTER 8

GENERAL DISCUSSION

This thesis describes a study on diet choice, foraging behaviour and the effect of predators on feeding in three-spined sticklebacks. Detailed results are presented and discussed in the appropriate sections of this thesis. In this chapter, the main findings of the previous chapters are summarized and discussed in general terms.

Analyses of the behaviour of sticklebacks feeding in laboratory tanks on freely available prey of different sizes (Pages 37-39) have shown that:

a. Variation in fixation times correlates with that of handling times.
b. Handling time is an increasing function of the amount of food in the stomachs.
c. Both fixation and handling times differ from one prey type to another.
d. Both fixation and handling times are an increasing function of prey size and a decreasing function of fish size. These patterns of variation in handling time are in agreement with that of other studies on different fish such as sunfish (Werner 1974) and fifteen-spined sticklebacks (Kislalioglu and Gibson 1976 a).

The profitability value of Daphnia (zooplankton) and Tubifex (benthos) increases with prey size (Page 47). This trend has been described before by many studies on planktivorous fish (e.g. Werner and Hall 1974, Gardner 1981) and is suggested as the explanation of the preference of many fish species for larger prey. However, the converse is found to be the case for other, larger, benthic prey such as Asellus and the larger sizes of Chironomid larvae, where the profitability value decreases with increase in prey size. This

indicates that the energy gain from increasing prey size can be overriden by the cost of increasing handling times for larger prey.

Sticklebacks with full range of experience with natural prey are more efficient feeders than inexperienced sticklebacks (Pages 55-56). When feeding on natural prey, experienced sticklebacks respond faster, spend less fixating and handling times and require fewer attacks to the prey before eating them. Such experience can be acquired in the laboratory after just few days of feeding on natural prey. Location and identification of the prey depend on the specific stimulus features such as movement, colour and shape (Hyatt 1979). It is likely, therefore, that the faster response of experienced fish is the outcome of learning to associate such characteristics with food through previous encounters in nature. Many studies have reported an improvement in feeding efficiency in animals with increased experience. Colgan et al. (1986), for example, have reported that experienced large mouth bass (Micropterus salmoides) perform more bites at prey than inexperienced bass. Winfield et al. (1983) have reported an increase in the number of approaches resulting in successful captures as bream (A. brama) become experienced with the prey. Such increases in feeding efficiencies of these two fish species are probably the result of experience-mediated improvement in detecting, fixating, handling and/or grasping the prey.

Sticklebacks collected from Loch Lomond, where both zooplankton and benthos are predominant, achieve higher net energetic return (joules/sec) from feeding on zooplankton than sticklebacks collected from Balmaha Pond where only benthos is predominant (Page 64). In contrast, sticklebacks from Balmaha Pond achieve higher net energetic return from feeding on benthos than Loch Lomond fish (this is not

significant). However, fish from both sites (and especially those from Loch Lomond) are more efficient at feeding on zooplankton than at feeding on benthos. These differences could be the result of experience with different natural foods and/or because fish from these two sites have different inherited morphological adaptations. Planktivores are known to use a suction process to capture individual prey items (O'Brien 1979, McComas and Drenner 1983). The higher the suction pressure attained, the higher the probability of prey catching and hence the greater the efficiency of feeding. Suction pressure is influenced by mouth size, with a small mouth governing a greater suction pressure than a large one. It is interesting to note that, in agreement with this pattern, Loch Lomond fish (which feed more efficiently on zooplankton) have narrower mouth than Balmaha Pond fish (Page 186). These differences in feeding efficiency clearly indicate that sticklebacks from different locations may have quite different predicted optimal diets. These observations reinforce the validity of the criticism made by Dill (1983) in that classical optimal foraging models do not accurately predict diet of animals unless they take into account the changes in behaviour due to learning.

The profitability value of the prey (determined as the energetic benefit/handling time) does not govern diet selection of sticklebacks choosing between prey items of various species under laboratory conditions (Page 79). The fish occasionally select the less profitable of the two prey items. This result agrees with that obtained from redear sunfish (L. microlophus), where the fish fail to select the most energetically rewarding prey (Stein et al. 1984). However, it does not agree with that obtained from fifteen-spined stickleback (S. spinachia) where, in the field, the fish select the size ranges of prey predicted in the laboratory to be optimal

(Kislalioglu and Gibson 1976 a). Stein et al. have attributed the deviation of their results from the theoretical predictions and from Kislalioglu and Gibson's result to the narrower difference in cost (time)/benefit ratio of the prey offered to their fish (1-3 sec/mg dry weight), which is much lower than that in Kislalioglu and Gibson's study (30 sec/mg dry weight). Thus, as the difference in cost (time)/benefit ratio becomes larger, predators become willing, or able, to discriminate between the prey more easily and the advantage of doing so may increase. My result may be explained, at least partly, in this way, since the difference in the profitability between any two prey offered to the sticklebacks has not exceeded 6 sec/mg dry weight. According to this interpretation, predators may have a threshold difference in cost (time)/benefit ratio for any two prey items below which active choice of the more profitable prey may not be worthwhile. This is in contrast to classical Optimal Foraging Theory which predicts that predator always should select the most profitable prey, whatever the difference in such ratio is.

Studies of the preference of sticklebacks for various visual stimuli of the prey (i.e. pattern of movement, speed of movement, colour, shape and size; Pages 89-90) have shown that sticklebacks do not differentiate between smooth and jerky movements. Considering the role of speed of movement, faster moving prey (up to 7.14 cm/sec) are preferred to slower prey and an optimal speed between 3.56 and 7.14 cm/sec is found. This preferred speed is higher than that reported by Kislalioglu and Gibson (1976 b) for fifteen-spined sticklebacks and by Meesters (1940) for different populations of three-spined stickleback. As far as colour is concerned, sticklebacks prefer red, pale, and dark coloured prey in decreasing order. This colour preference is not thought to be entirely a result of the contrast against the

background. Instead, it seems to be a true preference for prey reflecting light of a certain wavelength; this may be inherited or may be a result of experience with red prey in their natural habitats. Hafen (1935) has reported that Phoxinus laevis prefer yellow and green prey to red prey but after feeding them on red meat they prefer red prey. Regarding prey shape, straight, rectangular and globular shaped prey are preferred in decreasing order indicating that worm or larvae-like shape is the preferred shape. Again this shape preference may reflect the habit of the fish of feeding on worms and larval food in their natural habitat. Meesters (1940) has found that three-spined sticklebacks respond maximally to the shape of wavy thread which is close to the shape of the natural food of the fish. Concerning size preference, larger prey was preferred to smaller prey.

A comparison between the relative strengths of various dimensions of the prey stimuli in determining prey choice (Pages 93-94) shows that when the prey is red, colour is a stronger determinant than movement followed by shape and then by size (i.e. colour > movement > shape > size). When the prey is pale, movement becomes a more important determinant than colour which is followed by shape and size (i.e. movement > colour > shape > size). The role of movement as the most important determinant of prey choice (when no red colour is involved), agrees with other studies on other species of fish (eg. turbot, Scophtalmus maximus, Holmes and Gibson 1986). However, this result is no longer in agreement when red colour is involved in the comparisons. So, the red colour of a prey is indeed the strongest stimulus in determining prey selection of sticklebacks.

The identified preference of three-spined sticklebacks in terms of profitability of potential prey and visual characteristics can be

integrated (Pages 100-101). The fish tend to select the prey which possess one or more preferred visual feature(s). Thus, sticklebacks feeding in the laboratory use simple rules to select their prey. These rules in some cases lead them to the more profitable prey item and in other cases do not do so. Thus, in respect of these laboratory results, classical Optimal Foraging Theory, which predicts that animals should always choose the most profitable prey items, serves as a simple, testable, initial hypothesis but does not accommodate the full complexity of foraging behaviour in animals. Many authors have also launched similar criticisms on the simplicity of this theory and more recent models attempt to take these into account (eg. Krebs et al. 1980, Pulliam 1980, Krebs and Davies 1987).

A survey of the potential food of three-spined sticklebacks in Loch Lomond (Pages 117-119) has shown that this food is variable in space (even within few yards) and in time (from month to month within a single season). Vegetated and stony substrates provide higher availability of both zooplankton and benthos than sandy substrate. The abundance of zooplankton is high during June and decreases through to August, whereas the abundance of benthos shows the converse trend. This variability indicates, firstly, the need for sticklebacks foraging in nature to make decisions about which patch to feed in, secondly, the complexity of such decisions and, finally, the need for the information about habitat profitability to be continuously updated.

Sticklebacks select zooplankton prey and certain active prey of benthos (Page 145). This coincides with a selection of actively moving, red and exposed prey types. Therefore, sticklebacks feeding in the wild, like those feeding in the laboratory, use simple rules to

select their prey. However, unlike the situation in the laboratory, in the natural environment these rules lead the fish to the more profitable prey type since feeding on zooplankton (which have the visual features which are preferred by sticklebacks) is more profitable than feeding on benthos (which generally lack these features); see chapter 3 (Page 64). Thus, the 'rules of thumb', that lead sticklebacks to a diet that approximate the optimum, have been achieved under these more natural conditions. It seems therefore that sticklebacks feed optimally under field conditions, but when under artificial conditions, their feeding may deviate from optimal.

The diet of sticklebacks varies both in time and in space (Pages 134-145). Although most previous studies on feeding in fish have concluded that food availability is the main factor in determinig prey selection, the results discussed in chapter 5 have shown that food selection in sticklebacks is more complex. Thus, it is influenced by the type of the substrate above which the food is distributed and by prey conspicuousness, motion, colour and escape ability. Thus, when evaluating food selection of sticklebacks, the contribution of prey accessibility should be taken into account and a distinction should be made between an active choice (resulting from fish preference for the prey) and a passive choice (resulting from differences in prey accessibility). Furthermore, interpretation of prey selection on the basis of the energetic contents of the prey will not be complete without taking into the account differences in prey accessibility.

Prey selection of sticklebacks facing a choice between zooplankton and benthos under natural conditions is influenced by the availability of these two food types. The fish are more selective (i.e. prey diversity and evenness indices of their food are low) during June when

the availability of the preferred food (zooplankton) is high. However, as the density of such food declines, sticklebacks broaden their diet and include the less preferred food (benthos) and thus prey diversity and evenness indices become higher. This behaviour is in accordance with the prediction of Optimal Foraging Theory (Pulliam 1975, Pyke et al. 1977). Schluter (1981) has outlined some predictions of Optimal Foraging Theory and concluded that the model that forager will generalize as food abundance diminishes is successful in predicting diets under controlled conditions and/or simple environments but not in complex, multifood type systems. My result does not support Schluter's conclusion but reinforces the outlined prediction of Optimal Foraging Theory. A result similar to that obtained in my study has been obtained by Horn (1983) who found that two herbivorous fishes (Cebidichthys violaceus and Xiphister mucosus), feeding in the field, broaden their diets during periods of reduced food supply.

The size of prey has long been considered to be the primary characteristic which is responsible for prey selection by planktivores since larger prey usually are more profitable. In the present study of prey size selection, sticklebacks feeding in the field (Page 153) show a tendency to select larger individuals of zooplankton (Bosmina coregoni and copepods) but this selection does not seem to be as strong as that reported by other studies (eg. Werner and Hall 1974). In the case of benthos (Chironomid larvae, Chironomid pupae and Eurycercus lamellatus), sticklebacks feeding in the field select smaller individuals even though these are less profitable. This field result does not entirely agree with that obtained from the laboratory because, in the laboratory tests (Chapter 4), sticklebacks have been observed to choose the larger prey (eg. Chironomid larvae)

preferentially. This suggests that factors other than size may be responsible for prey selection under natural conditions.

Comparative analyses of the diet of three-spined and the coexisting ten-spined sticklebacks feeding above a vegetated substrate in Loch Lomond during August 1985 (Page 157) suggest that no competition should occur between these two species since they eat different food types and show a positive selection for different prey. Even when these two species have similar diets (eg. Delbeek and Williams 1987), the segregation into separate 'niches' (three-spined sticklebacks inhabit the open water and ten-spined sticklebacks inhabit the dense vegetation) may function to reduce competition. Thus, food selection by three-spined sticklebacks is unlikely to be altered by the presence of ten-spined sticklebacks, at least in summer when food is abundant.

Analyses of the stomach contents of fish caught in Loch Lomond and in Balmaha Pond (Pages 171-172) have shown that Loch Lomond fish feed predominantly on zooplankton and Balmaha Pond fish feed predominantly on benthos. These diets reflect the type of food available in the natural habitats of these two fish populations (Page 171). However, detailed comparisons between the proportion of food eaten and that available in the habitat have suggested that both Loch Lomond and Balmaha Pond fish positively select zooplankton rather than benthos. This indicates that Balmaha Pond fish were forced to eat benthos as the only sufficiently abundant food and not as the preferred food. Thus, given that feeding on zooplankton is more profitable than feeding on benthos (Page 64), the benthic-feeding habit of sticklebacks from Balmaha Pond does not deter them from selecting the most profitable food, zooplankton.

Even though feeding in the water column (which has the profitable and the preferred food; i.e. zooplankton) is more profitable than feeding on the lake bed, Balmaha Pond fish feeding in Loch Lomond still spend more time on the lake bed than Loch Lomond fish (Page 181). Paszkowski and Tonn (1983) have shown that fish select their foraging areas on the basis of naturally preferred sites and not on the basis of immediate profitability. Thus, the preference for a habitat on the basis of its current profitability can be overridden by the preference for the general type of foraging location itself. In agreement with this, Balmaha Pond fish naturally inhabit dense vegetated habitats whereas Loch Lomond fish inhabit open habitats (Pages 168-169).

Studies of the morphology of feeding apparatus of Loch Lomond and Balmaha Pond fish (Page 186) have shown that Loch Lomond fish have narrower mouths and more numerous, closely spaced and longer gillrakers than Balmaha Pond fish. These differences in the morphology, as well as the differences in the feeding habit and habitat use (see above), suggest that Loch Lomond fish resemble 'limnetic' sticklebacks whereas Balmaha Pond fish resemble 'benthic' sticklebacks (Larson 1976). Loch Lomond fish are morphologically better suited to feeding on zooplankton than Balmaha Pond fish which are better suited to feeding on benthos. Nevertheless, both fish groups have shown a preference for zooplankton.

A major aim of this thesis is to investigate the effect of both predators and local predation pressure on feeding behaviour, and the effect of predators on habitat use in sticklebacks. The results discussed in chapter 7 have shown that in the presence of a perceived predation risk, sticklebacks tested in laboratory tanks (Pages

200-201) become slower to respond to the food and cease discrimination in favour of the more profitable of two prey types. Similarly, in field tests (Pages 208-209), sticklebacks feeding in the presence of the predator eat less, feed on different food type (which requires shorter handling time) and eat smaller prey items than fish feeding in the absence of the predator. These changes in diet result in a lower energetic intake but the reduced handling time mechanism and the indiscrimination in favour of larger prey items may increase the time available for vigilance. Fish are able to survive long periods of starvation (Brett and Groves 1979) and thus the cost of a short-term reduction in energy intake in favour of vigilance may be far outweighed by the benefit of avoiding predation.

Studies of the estimated predation risk at the study sites and deduction from the morphology of defensive apparatus (Page 187) suggest that sticklebacks from Loch Lomond and Balmaha Pond undergo a high and a low predation levels respectively. Behavioural investigations (Pages 208-209) suggest some difference in the behaviour of these two fish groups in response to predation risk. Although, in the presence of the predator, sticklebacks from both sites eat less food, only Loch Lomond sticklebacks show an additional change in their diets, i.e. they eat different prey types and smaller prey sizes. This differential behavioural modifications in response to predation risk may be because Balmaha Pond fish are exposed to lower predation risk or because their diet consists of small prey (Bosmina, see above) anyway. However, because only two populations are studied, the possible conclusion that the behavioural differences are an adaptation to local predation levels must remain tentative.

Predation risk causes sticklebacks to increase the use of the lake bed and avoid the water column (Page 216). This shift is similar to that described for bluegill sunfish (L. macrochirus) which shift their foraging location, in the presence of the bass (M. salmoides), from open water to vegetation (Werner et al. 1983). However, unlike bluegills, which also shift to different food types in the presence of a predator, sticklebacks still feed on zooplankton whether the predator is present or not. In presence of the predator, sticklebacks therefore may either capture zooplankton from just above the vegetation or make regular short feeding-visits to the water column.

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